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HPA Axis Dysfunction In Treatment Resistant Affective Disorders

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**HPA Axis Dysfunction
In Treatment Resistant Affective Disorders**

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ABSTRACT

Background: TRD (Treatment Resistant Depression) patients have been shown to have hypercortisolemia and a hyperactive HPA (hypothalamo-pituitary-adrenal) axis. The Cortisol Awakening Response (CAR) is a naturalistic measure of the HPA axis activity. Although found to be elevated in depression, it has never been explicitly studied in TRD; furthermore, results have never been compared between Treatment Resistant Unipolar Depression (TRUD) and Treatment Resistant Bipolar Depression (TRBD). In addition, dehydroepiandrosterone (DHEA), the other main adrenal steroid, and which may counteract the effects of cortisol on the brain, has never been measured in TRUD or TRBD.

Aims and Methods: To assess the state and relevance of HPA axis changes in treatment-resistant depression using the following methods: (a) salivary cortisol, DHEA and the ratio of Cortisol/DHEA, measured at several points of the day over 2 days; and (b) the CAR (AUC_g and AUC_i), measured over 2 days. These parameters were compared: between TRUD and TRBD; between patients in episode and in remission; and with matched healthy controls.

Results: TRUD patients in episode had a higher CAR (AUC_g) compared to controls, remitted patients and TRBD. They also exhibited hypercortisolemia throughout the day (AUC_g), and on some measures an elevated Cortisol/DHEA ratio. TRBD patients in episode exhibited a lower CAR (AUC_g and AUC_i) than controls, remitted patients and TRUD, particularly on Day 1. The Cortisol/DHEA ratio was also lower than controls on

some measures. However, patients with remitted TRBD had higher Cortisol/DHEA ratios (but not CAR) than controls.

Conclusions: The HPA axis is disrupted in treatment resistant affective disorders, but in opposite directions in bipolar patients, who are characterised by hypocortisolemia, and unipolar depressed patients, who show hypercortisolemia. These abnormalities are present during episode, but not in remitted patients, suggesting a state dependent change. These findings have implications for diagnosis and treatment.

Abbreviations

AUCg: AUC with respect to ground

AUCi: AUC with respect to increase

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic Hormone
Acetyl CoA	AcetylCoA
ADU	Affective Disorders Unit
ANOVA	Analysis of Variance
AUC	Area Under the Curve
AVP	Arginine-Vasopressin
BAD	Bipolar Affective Disorder
BD	Bipolar Disorder
BDI	Beck Depression Inventory
BHS	Beck Hopelessness Scale
BMI	Body Mass Index
BPD	Borderline Personality Disorder
CAR	Cortisol Awakening Response
CBG	Corticosteroid-Binding Globulin
CECA-Q	Childhood Experience of Care and Abuse Interview
CNS	Central Nervous System
CRF	Corticotropin Releasing Factor
CSF	Cerebrospinal Fluid
DHEA	Dehydroepiandrosterone
DHEA-S	Dehydroepiandrosterone-sulfate
DHEA(S)	DHEA and DHEA sulfate
DSM	Diagnostic and Statistical Manual of Mental Disorders
DSM-III-R	Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised
DST	Dexamethasone Suppression Test
ECT	Electroconvulsive Therapy
ELS	Early Life Stress
GABA	γ -aminobutyric acid
GAD	Generalised Anxiety Disorder
GCF	Gingival crevicular fluid
GC(s)	Glucocorticoid(s)
GDS	Geriatric Depression Scale
GR(s)	Glucocorticoid Receptor(s)
HADS	Hospital Anxiety and Depression Scale
HAMD	Hamilton Rating Scale for Depression
HPA	Hypothalamo-Pituitary-Axis
ICD-10	International Classification of Diseases-10
IDS	Inventory of Depressive Symptoms
LC	Locus Coeruleus
(L)-T4	(Levo)-Thyroxine
MADRS	Montgomery Asberg Depression Rating Scale
MDD	Major Depressive Disorder
MMSE	Mini Mental State Examination
MR(s)	Mineralocorticoid Receptor(s)
NE	Norepinephrine
OC(s)	Oral contraceptive(s)
OCD	Obsessive Compulsive Disorder

PD	Personality Disorder
POMC	Propiomelanocortin
PSQI	Pittsburgh Sleep Quality Index
PTSD	Post Traumatic Stress Disorder
PVN	Paraventricular Nucleus
P450 _{scc}	Cholesterol side chain cleavage
RLC	Recent Life Changes
SAD	Seasonal Affective Disorder
SCID-I	Structured Clinical Interview for Axis-I Disorders
SCN	Suprachiasmatic nucleus
SLAM	South London and Maudsley NHS Trust
SSRI(s)	Selective Serotonin Reuptake Inhibitor(s)
T3	Triiodothyronine
TRD	Treatment Resistant Depression
TRUD	Treatment Resistant Unipolar Depression
TRBD	Treatment Resistant Bipolar Depression
UD	Unipolar Disorder
Vs	versus

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CHAPTER 1: INTRODUCTION

BACKGROUND/OVERVIEW

There has been a long history of research into the role of the HPA axis in the aetiology of psychiatric disorders, stemming in part from its role as the main long term biological mediator of the stress response and the observed links between life stress and depression (Meyer et al., 2001). Research has focused primarily on the role of the adrenal steroid cortisol and abnormalities such as hypercortisolemia and non-suppression to the dexamethasone suppression test (DST) have been found in depression (Gastpar et al., 1992). Until recently however the role of dehydroepiandrosterone (DHEA), the other main adrenal steroid has received little attention.

From a diagnosis perspective, although research has extensively focused on depressive disorders, very few studies to date have differentiated or attempted to measure the above biological parameters in clearly defined Treatment Resistant Affective Disorders. Some studies have even included both subjects with Unipolar Depression, Bipolar Depression and Bipolar Disorder within one illness group which is clearly problematic. It is well known that distinctive differences between the two types of depression (Unipolar and Bipolar Depression) exist in gender, sleep and appetite symptoms, activity level and other characteristics such as the duration of symptoms and comorbidity. A recent study by Forty and colleagues showed that there are important clinical differences between the groups and that characteristics that best predict Bipolar rather than Unipolar Depression are: the presence of psychosis, diurnal mood variation and hypersomnia, excessive self-

reproach, diminished energy levels and low libido (Forty et al., 2008). A lot of these characteristics are also encountered in atypical depression which has been shown to exhibit a very different endocrine profile compared to Unipolar Depression (hypocortisolemia as opposed to hypercortisolemia) (Juruena et al., 2006; Gold et al., 1999; Sanchez-Gistau et al., 2009, Anisman et al., 1999). Therefore the clear definition of the above conditions when conducting research is of paramount importance, as it could introduce systematic error due to disease misclassification with conclusions that may be misleading or even wrong.

Physiology of the HPA Axis

The HPA axis is one of the most important endocrinological systems. In this section, the physiology of the axis is briefly described.

Hypothalamus/Pituitary

CRH

The hypothalamus and pituitary serve as the body's primary interface between the nervous system and the endocrine system. Activation of the axis involves release of CRH (Corticotropin Releasing Hormone) from the hypothalamus into the portal venous circulation, and synthesis of proopiomelanocortin (POMC), the precursor of ACTH, which is released from corticotroph cells. Therefore CRH stimulates POMC transcription and ACTH biogenesis, but also stimulates the release of ACTH. The release of CRH is controlled by a complex mechanism involving reciprocal inhibitory and stimulatory connections. CRH is secreted through CRH-R receptors but an autoregulatory negative feedback system exists inhibiting the above secretion via

inhibition of the relevant presynaptic receptors (Calogero et al., 1988; Aghajanian et al., 1983).

ACTH

ACTH is produced from POMC and released in a circadian way from corticotrophs in the human pituitary constituting 15-20% of the cells of the anterior pituitary. Apart from CRH the release of ACTH is influenced by numerous other factors, amongst which are oxytocin and Arginine Vasopressin (AVP), Vasoactive Intestinal Peptide (VIP), Atrial Natriuretic Peptide (ANP), opiates, the endocannabinoid system, catecholamines, Growth Hormone Releasing Hormone (GHRH), angiotensin, ghrelin, interleukins and glucocorticoids.

Circadian rhythm

The above circadian rhythm is mediated via the supra-chiasmatic nucleus (SCN) with an autoregulatory negative feedback system (Dunlap, 1999) and with AVP being another factor responsible for the modulation of ACTH pulses (Ur et al., 1995).

ACTH is the principal regulator of cortisol production by the adrenal cortex, the final part of the HPA axis. ACTH enters the systemic circulation and binds to specific high affinity receptors located on the surface of adrenal cortical cells and the skin. In addition to its role in regulating cortisol production, ACTH is also of importance in the production of aldosterone and adrenal androgens.

Adrenal Cortex

The adrenal cortex is the final part of the HPA axis. It is devoted to the synthesis of corticosteroid hormones from cholesterol. Other cortical cells produce androgens such as testosterone, while some regulate water and electrolyte concentrations by secreting aldosterone. In contrast to the direct innervation of the medulla, the cortex is regulated by neuroendocrine hormones secreted by the pituitary gland and hypothalamus, as well as by the renin-angiotensin system (Davies et al., 1992; Wan et al., 1996).

The adrenal cortex comprises three zones, or layers. Beneath the adrenal capsule the following adrenal zones can be distinguished. The zona glomerulosa is the outermost layer; it comprises 15% of the adrenals and is the main site for production of mineralocorticoids, mainly aldosterone, regulated mainly by angiotensin II, potassium and ACTH.

The zona fasciculata, situated between the glomerulosa and reticularis, comprises 75% of the adrenals and is responsible for producing glucocorticoids, chiefly cortisol but also corticosterone in humans. The zona fasciculata secretes a basal level of cortisol but can also produce bursts of the hormone in response to adrenocorticotrophic hormone (ACTH) from the anterior pituitary.

The zona reticularis the inner most cortical layer, produces androgens, mainly dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEA-S) in humans but also contributes to some glucocorticoid production (cortisol and corticosterone). This zone is evident after a year of life.

The adrenal medulla is the core of the adrenal gland, and is surrounded by the adrenal cortex.

Feedback mechanism

Adrenal steroidogenesis is regulated by two endocrine feedback circuits; these are the HPA axis (mainly glucocorticoids and sex steroids) and the renin-angiotensin-aldosterone system (mineralocorticoids). High cortisol blood concentrations suppress the activities of the hypothalamus and the pituitary gland and high intravascular blood volume suppresses renin action and lowers angiotensin levels. Suppression of the HPA axis may persist months following cessation of glucocorticoid therapy. This is also evident in some medical conditions such as Addison's Disease where there is ACTH hypersecretion as well as undetectable ACTH levels in cortisol secreting adrenal adenomas. Feedback inhibition is mainly mediated by the glucocorticoid receptor.

The HPA axis together with the sympathetic nervous system connects the brain with the periphery of the body. The CRH/AVP and LC/NE neurons and their peripheral axes are heuristically known as the stress system (Chrousos, 2009). The secretion of the end-product of the HPA axis, cortisol, is kept by an elaborate negative feedback system within an optimal time-integrated narrow range, which is quite stable in an individual subject (Chrousos, 2009).

Altered (increased or decreased) HPA activity has been reported in a number of conditions including Cushing Syndrome, Anorexia Nervosa, Obsessive–Compulsive Disorder, Diabetes Mellitus, Central obesity (metabolic syndrome), Post-Traumatic

Stress Disorder, Hyperthyroidism, Hypothyroidism, pregnancy, Adrenal Insufficiency, Chronic Fatigue Syndrome, Fibromyalgia, Rheumatoid arthritis, Asthma, Eczema.

Cortisol

Synthesis

Cortisol is synthesised via cholesterol derived either from the circulation from LDL and also produced de novo within the adrenal cortex from acetyl CoA. Cholesterol is initially transported to the inner mitochondrial membrane by the enzyme P450scc (cholesterol side chain cleavage), where it is converted to pregnenolone. It is then converted in the cytoplasm to progesterone by the enzyme 3 β -hydroxysteroid-dehydrogenase. Progesterone is then hydroxylated to 17-OH progesterone. From there 11-deoxycortisol can be produced and then cortisol by 11-hydroxylase. The zona glomerulosa cannot synthesise cortisol as it lacks the enzyme 17 α hydroxylase.

Secretion

Levels of corticotropin and cortisol peak in the early morning, fall during the day to reach a nadir at about midnight, and begin to rise between 0100 hrs and 0400 hrs.

A major part of circulating cortisol is bound to plasma proteins, mostly corticosteroid-binding globulin (CBG) (Siiteri et al., 1986). According to the free hormone hypothesis (Westphal, 1983), the bound fraction of cortisol is unavailable to tissues and only the unbound fraction (about 10% of total cortisol) is thought to be biologically active. Thus, CBG levels determine the actual amount of cortisol available to the organism. CBG, a 383-amino acid glycoprotein of hepatic origin plays a crucial regulatory role in the transportation of glucocorticoids to their target tissues and alterations in the circulating

or local tissue (e.g., adipose tissue) concentration of CBG closely reflect the bioavailability of glucocorticoids and their capacity to act on the glucocorticoid receptors of different organs and tissues. CBG production is controlled by a variety of factors (e.g., genetic variations, liver and thyroid function, nutritional status, inflammation, stress) and under physiologic conditions, its plasma levels are generally stable.

It has been shown that CBG synthesis is regulated by glucocorticoids (Cole et al., 1999) and that CBG concentration is influenced by circulating gonadal steroids (Kajantie and Phillips, 2006). CBG is stimulated by estrogens and consequently is higher in premenopausal women than men of similar age and increases with the use of oral contraceptives (Wiegratz et al., 2003). In particular, the use of ethinyl-estradiol containing oral contraceptives (OC) has been shown to stimulate CBG synthesis (Wiegratz et al., 1998). Studies have shown higher male bioavailability of cortisol as suggested by a study in 767 men and women showing higher salivary cortisol at 0800 and 1600 hrs in men (Brandtstadter et al., 1991). Following menopause levels of CBG drop. Therefore although the rise in ACTH in pre and post menopause is the same this is accompanied by a higher rise in plasma cortisol but not salivary cortisol due to the above reason.

Effects

The role of cortisol in human functioning has been extensively described, as it plays a necessary role primarily in homeostasis and mobilisation of energy (Chrousos and Gold 1998; Chrousos, 2009); its impact on other physiological systems and affective and cognitive functions has been also described (Chrousos and Gold 1998). Cortisol binds to

glucocorticoid receptors that are present in almost every tissue of the body.

Consequently, cortisol mediates many metabolic processes ranging from induction of mobilisation of energy, increasing cerebral perfusion rates and local glucose utilisation, enhancing cardiovascular output and respiration, redistributing blood flow, increasing substrate and energy delivery to the brain and muscles, to modulating immune function (McEwen and Seeman, 1999).

Exogenous glucocorticoids also have a role in pharmacotherapy and most often are used in the treatment of autoimmune, inflammatory and other medical conditions (Boumpas et al., 1993).

Receptors

One of the important mechanisms through which cortisol exerts its effects and one of the mediators of negative feedback regulating the actual levels of circulating corticoids, are the glucocorticoid and mineralocorticoid receptors. These are the two main cortisol receptors which are present in different parts of the brain including in the CRH neurons of the hypothalamus (Fuxe et al., 1985). The Type I or corticosterone (mineralocorticoid) receptors are found mainly in the neurons of the limbic organs predominantly the hippocampus and septum (McEwen et al., 1968) and work in modulating the response to environmental and emotional stimuli, with consequent changes in behaviour and HPA axis activity. These receptors reduce in number with age as the hippocampus loses 50 percent of its glucocorticoid type I binding sites (Sapolsky et al., 1986).

Type II glucocorticoid receptors on the other hand are mainly found in the CRH neurons of the hypothalamus but are also found in the brain areas that contain POMC, such as the hippocampus, lateral septum, amygdala, and nucleus tractus solitarius (McEwen et al., 1968) where they possibly participate in the behavioural, neuroendocrine and autonomic responses to stress (Sapolsky et al., 1986). Experiments in rats have shown that during stress, the occupancy of type I receptor changes only minimally, whereas that of type II receptor changes considerably (Reul et al., 1985).

Clearance

The circulating half life of cortisol varies between 70 and 120 minutes. Approximately 1% of cortisol is excreted unchanged via the kidneys (urinary free cortisol).

DHEA and DHEA-S

Synthesis

DHEA and DHEA-S are major secretory products of the adrenal cortex. DHEA was first isolated in 1934 from urine and in 1944 its 3 β -sulfate was isolated. In 1954 DHEA was extracted from human plasma. DHEA, 5-androsten-3 β -ol-17-one, is a C-19 steroid (carbon steroid) is also derived from cholesterol. It is classified with the adrenal androgens, androstenedione and testosterone. Cholesterol is converted to pregnenolone by the mitochondrial enzyme P450_{scc}. Pregnenolone is converted to 17-OH-pregnenolone by the enzyme P450_{c17} but in contrast to the metabolic pathway that cortisol follows it is then converted to DHEA by the same enzyme. DHEA-S the sulphated form (5-androsten-17-one-3-yl-sulfate) circulates in plasma and is the major secretory product of the adrenal gland. DHEA is converted to DHEA-S by the adrenal

sulfotransferase (hydroxysteroid sulfotransferase) prior to its release in plasma, also known as DHEA sulfotransferase. DHEA-S can be converted back into DHEA by steroid sulfatase. However although a very large proportion of the daily production of DHEA-S is converted to DHEA in men and women, only approximately 10% of DHEA is converted to DHEA-S. Adrenal suppression by dexamethasone leads to a greater than 90% decrease in circulating DHEA-S concentrations (Arlt et al., 1998). Moreover, patients with adrenal insufficiency have very low, to undetectable levels of serum DHEA-S despite intact gonadal function (Yamaji et al., 1987). Minute amounts are synthesised de novo from the brain (Kroboth et al., 1999).

The above pathway through which DHEA/DHEA-S is synthesised differs between humans and primates. So unlike humans who secrete DHEA/DHEA-S from their adrenal glands and gonads, rats and mice can only secrete and produce DHEA/DHEA-S from their gonads, due to their adrenal glands lacking P450c17 (Le Goascogne et al., 1991).

There is no feedback mechanism controlling DHEA secretion within 'normal' DHEA values (Labrie, 2010).

Secretion

DHEA is secreted at a rate of 4 mg/day (DHEA-S 7 to 15 mg/day). On a molar basis the concentration of circulating DHEA-S is up to 500 times higher than DHEA (Labrie et al., 1997). The half life of DHEA is estimated to be 15-30 min, with a metabolic clearance rate of 2000 l/day, while the half life of DHEA-S is much longer at 7-10hrs, with a metabolic clearance rate of 5-20 l/day.

The sulphated form of DHEA, DHEA-S has a very long half-life in the circulation and exhibits little circadian rhythmicity, a reason why DHEA-S is often preferably measured (Kroboth et al., 1999; Goodyer et al., 1996).

DHEA levels have been found to be higher in women compared to men (Sulcova et al., 1997) although equal levels between genders have also been reported (Labrie et al., 1997). So far it has not been shown that menstrual cycle has an effect on levels of DHEA which appear to be stable throughout the cycle (Vermeulen et al., 1980). DHEA/DHEA-S are precursors to both estrogens in pre and post menopausal women (up to 100% following menopause) and androgens in men (Labrie et al., 1997).

Although ACTH is also considered to be a stimulus for androgen production, it may not be the only stimulus. Adrenarche for example is not followed by increased ACTH or gonadotrophins (Genazzani et al., 1983). Such discrepancies between adrenal androgen and glucocorticoid secretion have led to a suggestion of an additional androgen stimulating hormone. Several factors have been suggested including leptin, however an additional stimulus that could cause DHEA secretion remains to be unknown (Hosoda et al., 2008). It is not clear whether the same rhythm or suprachiasmatic stimulus is responsible for the secretion of DHEA. There is no evidence that DHEA and DHEA-S exert feedback on the HPA axis (Salek et al., 2002).

Until recently DHEA-S has been found to be a more suitable marker than cortisol of individual function of the adrenal cortex because its long term stability is higher. 90% of DHEA/DHEA-S are bound to albumin and 3% are bound to sex hormone-binding globulin (SHBG). In contrast to the binding affinity of plasma proteins to

cortisol which is high, albumin has low affinity for steroids. Although DHEA is weakly bound to albumin, DHEA-S is relatively strongly bound to albumin (Ebeling et al., 1994).

DHEA/DHEA-S are converted to the potent androgens T and DHT in peripheral tissues predominantly in the prostate, sebaceous glands, the external genitalia and the hair follicles.

Effects

DHEA has predominantly androgenic effects. In females during the follicular phase of the menstrual cycle it accounts for two thirds of testosterone production. In humans excessive androgen production could be manifested as hirsutism, male baldness, menstrual irregularities, infertility.

Receptors

As opposed to cortisol which binds to its receptors in almost all tissues, a DHEA/DHEA-S receptor has not been yet identified.

Clearance

In terms of their clearance, the more abundant circulating concentrations of DHEA-S are due in part to the more rapid clearance of DHEA from the blood at a rate of approximately 2000 l/day, whereas DHEA-S clearance is about 13 l/day (Lephart et al., 1987; Longcope, 1996). The clearance rates of DHEA and its sulphate are also influenced by their protein-binding characteristics as discussed above.

Concentrations of Cortisol

Plasma

Cortisol has been extensively measured in plasma, urine and has also been measured in CSF. Single and multiple measurements have been used. In plasma and urine, 24-hr collections have been attempted to provide a stable measure of adrenocortical output. Plasma concentrations of single or multiple cortisol levels have also been extensively used. There is a large individual variation with normal morning plasma cortisol values ranging between 165-690 nmol/l (Guazzo et al., 1996). Plasma free and salivary cortisol do correlate; however, total plasma cortisol shows a non linear correlation with saliva, as the former depends on the relative saturation of CBG (Aardal-Eriksson et al., 1998; Vining et al., 1983). Due to more than 90% of plasma cortisol being protein bound, the results of the conventional assay are affected by drugs, oral contraceptive use or conditions such as pregnancy that alter CBG levels. Few laboratories have developed methods for the measurement of free levels of plasma cortisol.

Urine

Urinary free cortisol is an integrated measure of plasma free cortisol; as cortisol secretion increases, the binding capacity of CBG is exceeded, resulting in a disproportionate rise in urinary free cortisol. Normal values are less than 220 to 330 nmol/l in 24 hours depending on the assay used. It may not always provide a reliable measurement method of the HPA axis activity as urinary free cortisol represents only 2-3% of the daily adrenal cortisol secretion. Instead cortisol metabolites are routinely measured. Furthermore some measurement methods have large variability at the lower part of the analytical range (Jerjes et al., 2006)

Concentrations of DHEA (plasma, CSF, Brain)

Plasma

In humans, plasma DHEA concentrations are only found in the range of 1–4 ng/ml, but circulating DHEA-S concentrations are much higher on a molar basis, between 250 and 500 times higher in women and men, respectively (Barrett-Connor et al., 1986; Labrie et al., 1995).

In animals administration of CRH or ACTH leads to significant changes in plasma and brain DHEA levels but no such changes are observed in DHEA-S concentrations.

DHEA-S response to exogenous ACTH administration is smaller than that of DHEA (Sirianni et al., 2005).

CSF

DHEA in the CSF correlates with circulating DHEA (Kancheva et al., 2011; Guazzo et al., 1996).

Brain

DHEA has been termed a neurosteroid. Part of neurosteroids may be synthesised *de novo* in the CNS, but also steroid metabolites may be synthesised in the CNS or transported through the blood brain barrier from the periphery. Although the validity of levels of DHEA in brain has been recently questioned due to the method used (solvolysis) recent studies that used a different analytic method (gas chromatography mass spectrometry) still found high DHEA levels in human brain (Weill-Engerer et al., 2003).

DHEA can then be measured in the periphery from blood, plasma, urine, saliva and these levels correlate with those found in the cerebrospinal and ventricular fluid.

Cortisol and DHEA in relation to age

The production of cortisol does not decrease with age. On the other hand there is a gradual decline in DHEA/DHEA-S. Adrenal secretion of DHEA gradually decreases over time and this seems to be linked to a selective decrease in the number of functional cells of the zona reticularis. DHEA/DHEA-S are highest during the 3rd decade of life and then decline so that by the age of 70 values are about 20-30% of peak values apart from the 5% of the population that continues to exhibit high DHEA levels (Kalimi et al., 2000). Of importance is that in postmenopausal women nearly 100% of sex steroids are produced in peripheral tissues from precursors of adrenal origin.

Saliva Measurements and composition

Production of saliva

Most saliva is produced by three pairs of major salivary glands (parotid, submandibular and sublingual) and to a lesser extent by the numerous small buccal glands which line the mouth. It also contains a small and variable amount of plasma exudate from abrasions in the oral cavity and of gingival crevicular fluid which leaks out from the tooth-gum margin. Saliva is formed predominantly in specialised secretory endpieces often called acini or secretory tubules which make up the largest part of the salivary glands. The protein content of gingival fluid is similar to that of plasma and provides a potential route for the entry of many hormones into saliva. Therefore any substance present in plasma may be expected to be present in the whole saliva at a concentration of the order of 0.5% of its plasma concentration.

The Composition of Saliva

Saliva is predominantly a mixture of water and electrolytes which enter into the acini through capillaries that surround the salivary glands. The whole saliva is a mixture of the fluids secreted by all of the various saliva glands, and it may also contain other components such as bronchial and nasal secretions, fluid that comes from the junctions between gums and teeth (gingival crevice fluid or GCF), blood and serum from mouth wounds, micro-organisms and food debris.

The Control of Saliva Secretion and Composition

Saliva production changes throughout the day being higher during the waking hours, and diminishing greatly during sleep (Thie et al., 2002). Various stimuli including taste, smell, and chewing motions of the jaw greatly increase saliva flow (Thie et al., 2002; Lee and Turner, 1993). Salivary production is under sympathetic and parasympathetic control of the autonomic nervous system. The parasympathetic system is largely responsible for increases in fluid secretion by the salivary glands, but the sympathetic stimulation causes salivary glands to contract, increasing the flow of saliva (Edwards et al., 1997).

The Movement of Substances from Blood into Saliva

In addition to the organic compounds that are produced in the saliva glands some compounds pass from the blood stream in the saliva. These substances include drugs, hormones and proteins and it is their presence that has initiated research of the use of saliva as a diagnostic fluid. The advantages of salivary collection methods are also described later in this chapter.

The most common way for substances to migrate from blood to saliva is by passive diffusion through the porous capillaries surrounding the salivary glands. Materials can pass from the blood system into the space surrounding the glands, and then make their way directly through the membranes of acinus or duct cells. The ability of a molecule to diffuse passively through cell membranes depends partly on its size, and partly on how much electrical charge it carries, so if a molecule is polar in nature, or if it separates into charged ions while in solution, diffusion in saliva is difficult. Steroid hormones including cortisol are relatively small in size, and most of them are fatty, non-polar compounds, therefore they tend to pass relatively easily by diffusion. Other molecules such as the large protein hormones, or hormones or drugs that are bound to large carrier proteins while in the bloodstream, are too big to enter by this route (Vining et al., 1983).

Filtering through the tight spaces between acinus or duct cells is another way to enter in saliva. In order to do this the molecules must be relatively small. Sulfated steroids such as DHEA-S and estriol sulfate, which are not able to pass through the fatty cell walls because of their electrical charges enter principally by the filtration route. The correlation of salivary and serum concentrations of DHEA pre and post exercise suggests that even under exercise conditions the salivary values of cortisol and dehydroepiandrosterone can reflect the behaviour of these hormones in blood (Cadore et al., 2008).

Blood components can also gain entry into saliva from the outflow of the serum-like gingival crevicular fluid (GCF) from the gums, or from small injuries or burns in the mouth. GCF is the major route by which certain molecules, which would ordinarily be too large to pass by either diffusion or filtration, can find their way from serum into saliva (Vining et al., 1983).

Advantages of salivary hormone assays (concentrating on cortisol)

Measuring hormones in saliva has clear scientific advantages. These are summarised below:

1. It avoids stress related to venesection.

Stress may cause elevation of many hormones, particularly cortisol. Salivary sampling avoids the pain and apprehension that can very often be associated with venesection.

2. Samples may be collected from home.

Investigations requiring repeated sampling are easily undertaken at home. Furthermore samples can be stored for a limited period of time in the freezing compartment of a domestic refrigerator until delivery to the relevant laboratory.

3. Multiple samples are facilitated.

Where multiple samples of saliva are needed the need for a repeated venepuncture or the use of an indwelling cannula are avoided.

4. The time lag between changes in salivary and plasma cortisol is very short (1 to 2 minutes). Furthermore saliva flow has no impact on salivary cortisol concentrations.

5. Salivary levels reflect the free plasma fraction therefore there is a high correlation between plasma unbound (and physiologically active) cortisol and salivary cortisol.

The non protein bound fraction of a hormone reflects their biological activity. Therefore measuring the plasma protein level has many caveats as there are several factors which could potentially alter the level of binding proteins when measuring the total plasma steroid level. This problem is clearly overcome when salivary samples are utilised which measures free hormonal fractions.

6. Salivary samples facilitate studies of normal physiology.

The easy non invasive procedure of using salivary sampling will enable many studies of normal endocrine function that were previously impractical to take place.

7. Saliva is not considered a class II biohazard.

Unless visibly contaminated with blood, human saliva is not considered a class II biohazard (Centers for Disease Control) affording for researchers administrative and safety benefits (Granger et al., 1999).

8. Cost.

Cost is often less when salivary sampling is used compared to some other methods of cortisol collection.

Methods of salivary collection

In terms of salivary collection different techniques are available. Often the decision regarding the most appropriate collection method is based on the sample characteristics, frequency of collection and measuring additional substances which sometimes can be affected by certain collection methods. In adults cotton dental rolls mainly salivettes (Sarstedt, Nümbrecht, Germany) are often preferred. Salivettes have advantages over other collection methods as the cotton swab is impregnated with citric or similar acid which aids salivary flow. However due to aspecific binding swabs or interference by other substances such as phytoestrogens that may be present, cotton salivettes or swabs should not be used when other steroids such as DHEA are concurrently measured (Granger et al., 1999). An alternative method of salivary collection is by drooling directly or with the aid of a straw directly in unrecycled polypropylene tubes (IBL, 2006) to avoid absorbtion of the hormone by the material. A study by Strazdins and colleagues comparing salivettes to passive saliva drool concluded that the same amount of saliva can be collected faster by using salivettes (Strazdins et al., 2005). However often larger saliva volumes need to be collected by the use of salivettes as a significant amount of saliva could remain in the cotton after centrifuging (De Weerth et al., 2003).

In depressed patients, chewing on an inert substance such as parafilm or making chewing movements are ways for stimulation of salivary flow. Cortisol has shown to remain stable in room temperature for a week and has been shown to remain stable following multiple freezing cycles up to 270 days.

In summary whole saliva collected by passive drool has the following distinct advantages

- a. enables a large sample volume to be collected,
- b. minimises the influence of substances used to collect or stimulate saliva flow on immunoassays,
- c. produces a sample that can be assayed for multiple markers, and
- d. allows unused sample to be frozen in an archive for future assay without concern that there would be interference with those assay protocols. Of course this procedure requires a competent, compliant, aware (and awake), and capable research participant (Granger et al., 2007). This may be a disadvantage in saliva collection. This method might not be the most advisable for use in young children.

Validity of Salivary Cortisol Measures

Collection time

When measuring cortisol in saliva a very large intra-individual variation has been found between subjects depending on time of collection. In a study where cortisol in saliva was determined in matched samples of saliva and serum were collected at 0800 hrs and 2200 hrs from 197 healthy volunteers reference ranges, were estimated to 3.5-27.0 nmol/l at 0800 hrs and < 6.0 nmol/l at 2200 hrs reflecting the variation in total serum cortisol. Similarly intra-individual stability for 0800 hrs salivary cortisol samples has

been found to be low (Coste et al., 1994). This very large variation for a single point measurement at this time of day can be explained by the recent findings that awakening stimulates secretory activity in the HPA axis. Salivary free cortisol exhibits a pronounced response to awakening, which is paralleled by changes in ACTH and total serum cortisol (Wust et al., 2000a).

Although salivary cortisol has advantages over other methods of cortisol collection it has been found to be less repeatable than plasma cortisol. In the context of studies such as those of dexamethasone suppression, salivary cortisol measurements may only be appropriate for large numbers of subjects. A study by Reynolds and colleagues showed that following administration of a low dose DST in 29 healthy subjects, pre dexamethasone cortisol concentrations were far more variable for saliva than for plasma. Post dexamethasone, both measurements showed an approximately 30% suppression from baseline, but the variability of salivary cortisol was much greater. Patient numbers in our study were however not that small, thus variability between the 2 methods of collection may not be that large (Reynolds et al., 1998).

Circadian rhythmicity

Dorn and colleagues compared salivary and serum cortisol circadian rhythms by collecting hourly samples over 24 h and concluded that the two profiles were synchronous and that salivary cortisol could be substituted for serum cortisol for the assessment of circulating cortisol rhythmicity (Dorn et al., 2007).

Sample numbers/days

To comply with the day-to-day fluctuations in salivary cortisol mean values from two awakening samples of two consecutive days are recommended as done in previous studies (Goodyer et al., 2000; Halligan et al., 2004).

In summary, there is a considerable body of evidence to support the validity of salivary cortisol measures with strong correlations between salivary and serum free or total cortisol having been reported. Salivary cortisol is now used as a diagnostic/screening tool for Cushing's syndrome.

Salivary DHEA

DHEA has been measured in saliva and has been found to correlate with plasma levels. The reference range for DHEA using 50µl of saliva without extraction is 12.3-3000 pg/ml with the luminescence immunoassay and 10-1440 pg/ml using the ELISA immunoassay (ibl-hamburg.com). Although salivettes are often preferred in salivary collections they have been shown to interfere with DHEA levels (due to the citric acid) therefore their use in measuring DHEA is not recommended (Granger et al., 1999).

DHEA-S has also been measured in saliva (Vining et al., 1983). Although its concentration in pure parotid saliva was 3 nmol/l, in whole saliva it averaged 16 nmol/l and was highly variable (0.6-70 nmol/l), due to contamination of the saliva by plasma exudate from the gingiva or mouth abrasions. Furthermore, DHEA-S concentrations were markedly dependent on salivary flow rate. Conjugated, polar steroids such as DHEA-S that enter saliva via the tight junctions are almost excluded from saliva, therefore their measurement in saliva is problematic (Vining et al., 1983).

Cortisol Awakening Response (CAR)

What is the CAR?

Until the beginning of the 1990's measuring morning cortisol was done independent of the awakening time. Standard clock times such as 0800 hrs were used without taking into consideration individual awakening times. This introduced methodological bias (measurement bias) particularly given that depressed patients often suffer with sleep disturbances and wake up at much different times compared to controls. As a consequence results were inconsistent and difficult to interpret. Stimulation of the HPA axis (which provides more consistent results) was only evaluated via elaborate tests which were mostly undertaken under lab conditions (Dexamethasone, Prednisolone suppression test, Juruena et al., 2006). These tests are often still used as they provide information on HPA activity related to feedback mechanisms.

In the beginning of the 1990's it was noted that there is a brisk increase of cortisol release in the blood stream following awakening as a distinct phenomenon superimposed on the daily circadian rhythm of cortisol. This accumulating evidence that the CAR is a discrete part of the circadian rhythm was consequently extensively replicated (Schmidt-Reinwald et al., 1999; Wust et al., 2000; Edwards et al., 2001; Wolf et al., 2005). In healthy adults there is a rise of 50-160% in the first 30min following awakening (Clow et al., 2004). This increase ranges from 4-15 nmol/l. Pruessner and colleagues showed that the cortisol release in the blood stream is increased by about 38-75% and reaches a peak at approximately 30 mins following awakening (Pruessner et al., 1997). This is followed by a decrease after about an hour post awakening. Although initially thought that the CAR was part of cortisol's circadian rhythm, the above and other evidence showed that the CAR is a distinct phenomenon superimposed on the

circadian rhythm of cortisol as it adds a significant incremental effect to the linear trend of increasing cortisol concentrations in the early morning hours (Wilhelm et al., 2007; Chida and Steptoe, 2009). However it was not until 2007 that the CAR was measured under lab conditions and shown not be part of cortisol's circadian rhythm, but in fact a distinct phenomenon (Wilhelm et al., 2007).

The CAR is considered a reliable measure of the HPA axis activity (Schmidt-Reinwald et al., 1999) and has been studied extensively over the past two decades, not only in healthy populations, but also in relation to many disorders including cardiovascular, autoimmune, atopic, allergic, and psychiatric diseases, among others (Wust et al., 2000; Clow et al., 2004).

The function of the CAR is not entirely clear. The CAR seems to be related to anticipation of the demands of the day. This is supported by evidence that shows it is higher on weekdays compared to Sundays. Indeed the cortisol response to awakening was found to be lower on Sundays compared to the rest of the days ($p < 0.05$) when measured during 7 consecutive days in 28 medication free, full time employed individuals, working Monday to Saturday (Kunz-Ebrecht et al., 2004). Rohleder and colleagues examined the CAR in professional dancers prior to dance floor competition. CAR was found to be elevated on the day of the competition particularly in highly focused conditions of stress (couple vs group competition) a finding that according to the author supports 'the notion of a social self-preservation system that is physiologically responsive to threats to the social self' (Rohleder et al., 2007). However although the majority of studies have reported an elevation of CAR during weekdays compared to weekends (Kunz-Ebrecht et al., 2004; Schlotz et al., 2004; Thorn

et al., 2006), other studies have not showed such changes (Kudielka and Kern, 2004; De Weerth and Buitelaar, 2005). Findings of weekday/weekend differences were associated with higher levels of perceived stress, and lower feelings of control and happiness on weekdays in one study (Kunz-Ebrecht et al., 2004), but were not accounted for by differences in stress in another study (Thorn et al., 2006). Thus some authors have perceived the CAR more as an adaptive response, providing a ‘boost’ to meet anticipated demands for the day (Adam et al., 2006), whereas others have related anticipations of negative, stressful events with an elevated CAR (Kunz-Ebrecht et al., 2004; Scholtz et al., 2004).

Neural regulation of CAR

Since regulation of the CAR is via the HPA axis both the limbic system and the prefrontal cortex are involved. The hippocampus plays a central role. Thus, there is an abolished CAR following unilateral or bilateral hippocampal damage despite subjects having a preserved cortisol diurnal variation (Buchanan et al., 2004) as measured in 22 subjects with hippocampal damage, 7 patients with brain injury outside the medial temporal lobe, controls and caregivers. Participants provided saliva samples collected with salivettes over the course of a single day. These were given immediately upon awakening, at 30 minutes following awakening, at 0800 hrs, 1100 hrs, 1500 hrs, 1800 hrs and before bedtime.

The other brain area that affects the CAR is the suprachiasmatic nucleus (SCN) (Clow et al., 2004; Fries et al., 2009; Hucklebridge et al., 2005; Edwards et al., 2001; Thorn et al., 2010). The SCN, which acts as the biological clock of the body and is affected by light, directly projects to the PVN.

The intrinsic rhythms of the SCN are synchronised with the external day-night cycle by light, via the retinohypothalamic tract. Seasonal variations in day length and light are detected by the SCN, which makes adjustments in the organism's physiology. Thus, studies have shown that morning cortisol is higher (King et al., 2000) in winter although other studies have shown no seasonal variability (Lac and Chamoux, 2006) and others have shown that children have higher diurnal cortisol levels in summer (Matchock et al., 2007). In Seasonal Affective Disorder, (SAD), a form of atypical depression where patients complain of hypersomnia, extreme lethargy, overeating and carbohydrate craving (Sher et al., 1999), the CAR has been found to be attenuated in winter months (Thorn et al., 2011).

Based on the above evidence, it is likely that the SCN, is one of the major regulators of the CAR. This is different to the regulation of cortisol during the day (Clow et al., 2010). Cortisol during the day does not appear to be regulated or influenced by light (Scheer and Buijs, 1999; Leproult et al., 2001).

It is also of note that a significant genetic influence has been observed for the CAR but not for the secretion of cortisol during the day based on twin studies (Wust et al., 2000a).

Apart from the hippocampus and the SCN, other brain regions regulate the HPA axis activity and indirectly the CAR by directly innervating the PVN of the hypothalamus such as the hypothalamic subregions, the thalamus, the bed nucleus of the stria terminalis, the nucleus of the tractus solitarius and the raphe nuclei (Herman et al.,

2008). Other regions that indirectly regulate HPA axis activity and the CAR including the amygdala and prefrontal cortex (Herman et al., 2008).

Number of days of cortisol measurement

Research into the CAR is typically conducted using cross-sectional designs with data accumulated from 1 to 3 study days per person. While this is a sensible and effective approach to investigate stable, trait-like associations, recent evidence highlights the high amount of state specificity in measures of the CAR (Hellhammer et al., 2007) as also mentioned below. Although state variability is not necessarily a problem for cross-sectional research if random (that is, factors influencing state variability show no systematic associations with experimental groups or variables) it obviously needs to be taken into account during the design of a study to lessen confounding of results. Therefore here are presented factors associated with intra-individual variability in measures of the CAR.

Factors affecting CAR

The CAR is both under the genetic influence but also shows individual variability (Wust et al., 2000a; Hellhammer et al., 2007). To minimise confounding related to trait factors these are taken into account during the design of a study (see methodology and results chapter). Of note is that previous research has not always drawn conclusions as to whether particular factors do indeed have an influence on the CAR.

The initial factors that were measured in relation to the CAR involved age and gender. When age was measured results varied according to the sample size. Studies that used large sample sizes as those by Pruessner and colleagues (Pruessner et al., 2007) and

Wust and colleagues (Wust et al., 2000a,b) did not report any effects on CAR as opposed to those that used a smaller sample (Kudielka and Kirschbaum, 2003).

Similarly study results that describe differences in CAR between males and females vary with most studies reporting no differences in the cortisol rise between genders (Kudielka et al., 1998). Although some studies have reported females to have a larger CAR compared to men, gender seems to explain little of the variability measured in CAR (Wust et al., 2000a,b; Pruessner et al., 2007). Menstrual cycle and oral contraceptives have also been extensively measured, particularly given that the use of oral contraceptives has a direct effect on CBG. Pruessner and colleagues amongst others evaluated their effect which was found to be small (Pruessner et al., 1997). With regards to the menstrual cycle results are conflicting. Although some studies report that the salivary cortisol rise does not differ between menstrual phases (Kudielka et al., 2003), other studies report that salivary responses differ significantly between women in the luteal or follicular phase even when using the TSST psychological test (Kirschbaum et al., 1999).

Physical factors such as obesity have also been shown to be affecting CAR. Although men with visceral obesity exhibit an enhanced CAR this seems to return to normal in a reduced obese state. In women although peripheral fat accumulation does not modify the CAR, it is increased during weight loss (Therrien et al., 2007). Upper respiratory disease increases the CAR (Edwards et al., 2003) whereas the CAR has been reported to be reduced in hypertension (Wirtz et al., 2007), atherosclerosis (Hurwitz et al., 2001) and functional gastrointestinal disorders (Bohmelt et al., 2005).

Another factor affecting CAR is burnout (emotional and physical fatigue). Generally burnout has been associated with low levels of morning cortisol and higher evening cortisol and a flatter diurnal secretory cycle (Morgan et al., 2002; Pruessner et al., 1999); however high cortisol on awakening has also been reported (Del Vente et al., 2003). Grossi and colleagues found differences in the CAR measured each 15 minutes for the first hour of the day between patients with high and lesser degrees of burnout without comorbid depression (Grossi et al., 2005). Melamed and colleagues also found that subjects with high chronic burnout had higher morning cortisol than subjects with low burnout but this was so only in cases of chronic and unrelenting/intractable burnout (Melamed et al., 1999). Other studies which only measured the morning cortisol rise between burned out and work engaged subjects did not report a significant difference (Langelaan et al., 2006).

Stress has been found to affect the CAR. Thus, Wust and colleagues demonstrated that the CAR was positively associated with higher levels of stress, worrying and social recognition (Wust et al., 2000b). In another study, middle aged women with high levels of stress had a lower morning cortisol rise compared to those with low stress levels (O'Connor et al., 2009). Chronic work overload has been associated with an increased CAR (Schlotz et al., 2004). De Vugt and colleagues found a greater CAR in caregivers with high stress levels compared to those with low stress levels (de Vugt et al., 2005). PTSD patients yield variable results either by showing an increase following awakening (Wessa et al., 2006) or no increase in CAR (Young et al., 2004).

Smoking has been measured in a multitude of studies and although most of these do not demonstrate a clear effect on the CAR, larger epidemiological studies report an

increased CAR in habitual smokers. Badrick and colleagues examined the effect of smoking on salivary free cortisol on awakening and daily cortisol release in 4231 subjects. Samples were collected on awakening, after 30 minutes and throughout the day. Smoking status was significantly associated with increased salivary cortisol release throughout the day which was also apparent when the CAR was examined separately. However, no differences were apparent between never-smokers and ex-smokers suggesting that smoking has a short term effect on the HPA axis. According to most studies it seems that the effect size of cigarette smoking on cortisol and the CAR is low (Badrick et al., 2007; Maninger et al., 2009). Although cortisol is decreased in subjects attempting to quit smoking the effect on CAR is not known (Ussher et al., 2006). Wust and colleagues assessed the effect of smoking and observed a slightly attenuated cortisol rise between smokers and non-smokers. The effect however appeared to be virtually negligible as it explained only 1% of the variability in the CAR (Wust et al., 2000b).

The effect of alcohol use on the CAR was evaluated in a cohort study of drinkers (Junghanns et al., 2007). Heavy drinkers (above 28 units of alcohol consumption per week in men and 21 units per week in women) had a greater rise in CAR compared to moderate drinkers (Junghanns et al., 2007).

Pain may have an effect on the CAR. However, a recent study found that back pain sufferers and healthy controls did not differ in their response to awakening, although there were significant interrelations between awakening responses and behavioural pain coping-strategies (Sudhaus et al., 2009).

Sleep related factors have been extensively studied in relation to CAR. These are discussed below:

Time of awakening has been extensively evaluated; results are conflicting with some studies reporting a higher CAR in subjects waking early compared to ones waking later (Edwards et al., 2001; Kudielka and Kirschbaum, 2003). Other studies however have not observed a significant impact of the time of awakening on the subsequent cortisol response (Pruessner et al., 1997; Wust et al., 2000b). Furthermore “morningness” and “eveningness”, human behavioural traits, have been shown to possibly affect the CAR as morning compared to evening chronotypes exhibit higher salivary CAR collected on 2 consecutive days (Kudielka et al., 2006). In contrast, neither mode of awakening (either spontaneously or by an alarm clock) nor waking at unexpected times seem to have an effect on the CAR (Wust et al., 2000b). In the same paper it was reported that sleep duration had a very small effect on CAR suggesting that subjects who report a shorter sleep length had a marginally larger CAR compared to the ‘long sleep’ group as assessed by performing a median split in the sleep time of the whole group (Wust et al., 2000a).

In order to assess whether there is an effect of sleep quality on CAR, the latter has been evaluated in subjects suffering with insomnia. Results are conflicting. Thus, although Backhaus and colleagues reported lower awakening response in insomniacs (Backhaus et al., 2004), other studies have not replicated these results suggesting that nightly awakenings do not have an effect on CAR (Dettenborn et al., 2007).

Exposure to light during the night has also been evaluated. Overall subjects exposed to light have a more pronounced CAR response (Scheer and Buijs, 1999; Thorn et al., 2009). On the other hand noise (low frequency exposure to noise) does not cause an awakening rise (Waye et al., 2003).

The CAR has not been found to be influenced either by social position or race, findings that have been supported by the Whitehall II and CARDIA studies (Kumari et al., 2010; Cohen et al., 2006).

Various reasons can impede participants from obtaining samples at the specific times requested (Kudielka et al., 2003). A delay in taking the first sample may result in an apparently blunted CAR as a result of the higher waking cortisol value. As a result, delays of ten minutes or more are considered “non-compliant” (Kunz-Ebrecht et al., 2004) and often these samples are excluded from analyses. Similarly, another study showed that delays of more than 15 minutes between wake time and collection of the waking cortisol sample can significantly affect CAR values (Okun et al., 2010). Waking should be defined as accurately as possible to standardise the assessment across participants (Adam et al., 2010). Common definitions are ‘when your eyes open and you are ready to get up’ or ‘as soon as you open your eyes and before your feet touch the ground’ (Cohen et al., 2006).

Non adherence to the study protocol has been often reported as a problem in measuring the cortisol response to awakening. Most of the studies rely on self report measures to determine adherence. Kupper and colleagues utilised simultaneous ECG and movement readings to explore actual times of awakening in relation to the shape of the CAR and

found that participants who did not show a rise in cortisol between their waking time and 30 minute later had woken up 42 minutes earlier than the time reported by them. The following criteria have been described by Thorn and colleagues: 'To be deemed adherent on any day participants had to show a rise (no matter how small) in cortisol concentration from sample 1 (immediately upon awakening) to sample 2 (15 minute post awakening) or sample 3 (30 minute post awakening)' (Thorn et al., 2006). Indeed using wrist actigraphy, an alternative way of controlling for subject compliance only a very small proportion of individuals do not show a positive CAR despite not delaying saliva sampling after waking (Dockray et al., 2008). The balance of evidence overall suggests that participants collect samples accurately in relation to objectively determined wake times (Kraemer et al., 2006; Dockray et al., 2008).

Calculation of the CAR

Studies have concentrated on measuring the CAR in two different ways. This is further mentioned under the statistical section of the methodology chapter. Methods include the following:

- 1) CAR_i or AUC_i (increase): This is the CAR calculated as the increase from baseline and so is independent of the actual cortisol levels on awakening. This measure of the CAR is related to the sensitivity of the system (Chida and Steptoe, 2009).
- 2) CAR_{auc} or AUC_g: The latter depends on the absolute cortisol levels which are present on awaking; if the waking cortisol levels are high, then the CAR_{auc} will be large even if the increase following waking is minimal. The AUC_g correlates with diurnal mean cortisol concentration (Edwards et al., 2001).

Recent evidence by Hellhammer and colleagues, following application of structural equation modelling to CAR measurements over 6 consecutive days, suggests that the CAR of a single day is determined to a great extent by state factors and only to a smaller degree by trait factors. This is particularly the case for the dynamic increase of the CAR with estimates of state specificity varying between 61% and 82% (Hellhammer et al., 2007).

From the above it seems clear that in order for research to be valid, a clear research protocol needs to be designed taking into consideration as much as possible all of the factors that could affect the CAR, to minimise the occurrence of systematic error and of positive associations which could be related to an independent factor. It also becomes clear that measuring the CAR becomes of greater value compared to single cortisol measurements, as secretion can be monitored over longer time periods, usually from 30-90 minutes. The CAR also decreases measurement bias, as there is no variability related to the time of awakening.

DHEA- what happens following awakening?

As opposed to cortisol there is no evidence of an awakening stimulatory burst of DHEA release. Hucklebridge and colleagues measured the salivary DHEA secretory burst following awakening in saliva in 24 medication free subjects (19 females and 5 males, mean age 24 years) over 2 days. Samples were provided via the drooling method immediately upon awakening and then at 15 minute intervals over the first 45 min following this. Using a 2-way ANOVA, no effects or trends were evident suggesting that DHEA levels do not show any consistent movement during the first 45 minutes following awakening. Absolute levels were comparable on the two days of testing

(Hucklebridge et al., 2005). This is the only study that has measured salivary DHEA following awakening.

Cortisol and Brain

At present there is much research that supports the presence of structural brain changes occurring in depressive disorder. The changes are particularly prominent in cortico-limbic areas (Ekstrand et al., 2008). Videbech and Ravnkilde performed a meta-analysis of hippocampal volume studies that were performed in unipolar depressed and bipolar patients showing that there is a reduction of hippocampal volume in Unipolar Depression. This same finding was not noted in Bipolar Disorder; however, compared to Unipolar Disorder, Bipolar Disorder is much less investigated (Videbech and Ravnkilde, 2004).

Cortisol and hippocampi size has been measured in Bipolar and Unipolar Depression. Higher levels of cortisol have been related to smaller volumes of hippocampi due to the putative toxic effect of cortisol on the hippocampus. Hippocampal volume in UD, BD and controls has been recently assessed in a meta-analysis of structural imaging studies (Kempton et al., 2011). In this study, 25 variables were used for comparisons. Most importantly the total hippocampal volume was also evaluated between groups. 225 studies were included in the comparisons. Compared with controls, patients with UD had smaller volumes of hippocampi. This fits well with the theory of hypercortisolemia in depression. The pituitary gland was also found to be increased which makes sense given that higher CRH levels have been found in depression (Nemeroff et al, 1998) and Arnone and colleagues found a trend for an increased pituitary volume in UD vs BD ($p=0.058$) (Arnone et al., 2011).

In remission patients had significantly larger hippocampal volumes compared to depressed patients ($p=0.04$) and there was no significant difference in volume between patients with remitted UD and controls. On the other hand patients with BD were not found to have reduced hippocampal volumes compared to controls, but on the contrary depressed patients were found to have reduced volumes of hippocampi compared to BD. Although hippocampal volume did not differ between patients with BD and controls, when age was taken into account patients with BD had an increased hippocampal volume compared to controls ($p=0.001$). One may therefore come to the preliminary conclusion that with age cortisol levels may change in the opposite direction in this group of patients compared to UD (thus hypocortisolemia as opposed to hypercortisolemia).

From the follow-up meta-regression analyses, there was no significant effect of patient age, sex, or medication use on any of the 6 regions in either BD or MDD studies.

To examine whether hippocampal changes are related to cortisol, O'Brien and colleagues compared 61 elderly depressed subjects who met DSM-IV criteria for Major Depressive Disorder and 40 healthy comparison subjects. Subjects underwent structural magnetic resonance imaging (MRI) and salivary cortisol assessment (over 3 days) with follow-up 6 months later. Subjects were hypercortisolemic at baseline. A reduction in the right hippocampal volume (6% decrease) was shown. This was not however associated with increased cortisol levels although it was significantly correlated with continuing memory deficits at 6 months (O'Brien et al., 2004).

However other studies have not arrived at the same conclusions regarding salivary cortisol and its relation to hippocampal volumes. Colla and colleagues reported that

patients who showed a subsequent decrease in cortisol under pharmacotherapy tended to have higher hippocampal volumes (Colla et al., 2007). Hippocampal shrinkage is seen in other conditions with hypercortisolemia such as Cushing Disease and in animal studies of cortisol treatment and psychosocial restraint. Memory dysfunction, a common finding in depression is seen in stressed or glucocorticoid treated animals and human subjects (Chrousos et al., 2009).

DHEA and Brain

DHEA has been greatly publicised due to its numerous proposed anti-ageing effects and effects in brain to the point that it has been considered by many a marker of ageing (Rudman et al., 1990). However the actions of DHEA/DHEA-S are still not fully understood.

DHEA/DHEA-S has positive effects on memory as shown by studies both in vivo and in vitro. Its effect on memory is thought to be due to the increased acetylcholine (ACh) release in the hippocampus as shown in anaesthetised rats (Rhodes et al., 1996). It is known that hippocampal ACh is closely associated with memory function (Gold and Chrousos, 2002) and that its increased release in the hippocampus can improve memory (Gold and Chrousos, 2002).

DHEA has been termed a neurosteroid since the early 1990's due to its ability to be synthesised 'de novo' from the brain (Baulieu and Robel, 1998). DHEA can be synthesised in vivo in rat and frog brains. DHEA can be synthesised in neurons and glial cells as well as in the zona reticularis of the adrenal glands (Baulieu and Robel, 1998). Although the role of DHEA is not entirely clear, what is clear is that it does

modulate the effects of other neurotransmitters; it increases the effects of the excitatory neurotransmitter, glutamate (Debonnel, 1993), and decreases the effects of the inhibitory neurotransmitter, GABA (Majewska, 1995) at the cell membrane exhibiting a neuroprotective effect in mice and rats (Fiore et al., 2004; Li et al., 2001). Its actions are also shown in vitro (Kaasik et al., 2001). When rat cerebral cortical cultures were subjected to anoxia for 2 hours in an anaerobic chamber and pretreated with DHEA/DHEA-S, an increased neuronal survival was noted. This increase in neuronal survival was not due to metabolism of DHEA/DHEA-S into estradiol, since concentrations of 17β -estradiol were not detectable in culture media. DHEA/DHEA-S protects against NMDA toxicity in fetal rat hippocampal cultures (Kimonides et al., 1999).

Hu and colleagues examined the effects of DHEA in rats against repeated immobilisation stress-induced weight loss, glucocorticoid receptor production, and lipid peroxidation. The stress test produced weight loss, an increase in adrenal weight, an increase in the number of glucocorticoid receptors and decreased plasma triglyceride levels. DHEA treatment of chronically stressed animals had no significant effect on plasma corticosterone, cholesterol, or triglyceride levels (25% decrease), which remained almost similar to those of chronically stressed animals. In contrast, DHEA given to control unstressed animals resulted in reduction in body weight, with a dramatic decrease in plasma corticosterone and triglyceride levels (50% decrease), when compared with control untreated animals. Thus, DHEA decreased triglyceride levels in both stressed and non stressed animals. However decrease in stressed animals was much greater and significant compared to that in non stressed animals (Hu et al., 2000).

Cardounel and colleagues showed that although treatment with glutamate increased the neuronal nuclear localisation of glucocorticoid receptors, this effect was reversed with DHEA treatment administered for 24 hours, suggesting that DHEA protects hippocampal neurons, at least in part, by its antiglucocorticoid action via decreasing hippocampal cells nuclear glucocorticoid receptor levels (Cardounel et al., 1999).

DHEA causes neuronal growth as it promotes lengthening of neuronal axons (Compagnone et al., 1998). DHEA's stimulation of embryonic cortical neurons causes a dose-dependent increase in calcium entry into cells an effect which is blocked by the NMDA receptor antagonists. This suggests activity-dependent neurosteroid synthesis and that DHEA may be synthesised and act locally to cause axonal growth in cortical embryonic neurons.

DHEA promotes neurogenesis and neuronal survival as shown in rats (Karishma and Herbert, 2002) possibly by affecting BDNF concentrations (Naert et al., 2007). Single injections of DHEA into adult male rats change regional brain concentrations of BDNF. Rats receiving DHEA had decreased BDNF content in the hippocampus, no change in BDNF content in the amygdala, and increased BDNF in the hypothalamus compared to sham rats. In vitro in human cortical brain tissue DHEA promotes survival of neurofilament positive, neuron-like cells (Brewer et al., 2001) and in human neural stem cells derived from fetal cortex it increases neurogenesis (Suzuki et al., 2004).

From the above it seems that actions of DHEA and cortisol in the brain are opposite to each other which is further discussed below.

Administration of DHEA

DHEA has been shown to have antiglucocorticoid effects in vitro and may protect against the adverse effects of raised circulating cortisol (Kalimi et al., 1994; Karishma and Herbert, 2002; Kimonides et al., 1998).

Effect on cortisol

In vivo even single administrations of DHEA have led to a reduction in cortisol concentration. Thus, Kroboth and colleagues measured cortisol concentrations during a 24hr period following administration of DHEA in a single blind placebo controlled crossover study. Once each morning, subjects took either placebo or 200 mg of oral DHEA. 24-hour DHEA and cortisol concentrations were subsequently measured. DHEA administration resulted in a decrease in plasma cortisol concentrations (mean, peak, and/or AUC) in healthy older women and men. The cortisol-lowering effect of DHEA was more pronounced in women than in men (Kroboth et al., 2003). The finding that single administrations of DHEA lead to a reduction in cortisol levels was replicated in a study by Wolf et al., (1997).

Effect on symptoms of mood-cognition

The potential for DHEA replacement and enhancement of cognition was tested in a study by Van Niekerk and colleagues, where participants received either 50 mg DHEA daily for 13 weeks, followed by placebo for 13 weeks, or the reverse, in a randomised double-blind cross-over trial design. Levels of salivary cortisol and DHEA were measured at 0800 hrs and 2000 hrs prior to each assessment session. Higher morning DHEA was associated with lower confusion, while higher evening DHEA was associated with lower anxiety and lower negative mood in the morning. Conversely,

higher morning cortisol and a morning Cortisol/DHEA ratio were associated with higher anxiety, general mood disturbance and higher current negative mood in the evening but also with higher confusion and lower visuo-spatial memory performance. Contrary to hypothesis, higher evening cortisol was associated with faster choice reaction time ($p=0.04$). Although these findings are consistent with an impairing effect of high cortisol on episodic memory and mood in older men, which may be attenuated by DHEA, when treatment effects were analysed, no significant effects of DHEA were observed on any of the trial outcomes, providing no support for benefits of DHEA supplementation for cognition or well-being in normal older men in the shorter-term (Van Niekerk et al., 2001).

The beneficial effects of DHEA administration on mood and cognition have also been reported. Alhaj and colleagues measured salivary cortisol and DHEA after administration of 150 mgs of oral DHEA twice daily over the course of a week as part of a double blind placebo trial. 24 male subjects who were not suffering from any major psychiatric conditions and had a cut off score of 8 on the BDI (Beck et al., 1961) were included in the trial. Salivary cortisol and DHEA were collected over 2 days using the drooling method. Following a post-hoc analysis it was shown that DHEA led to a reduction of evening cortisol concentration compared to placebo. DHEA following active treatment revealed a significant treatment effect ($p<0.0001$). DHEA was further shown to ameliorate mood (Alhaj et al., 2006).

Conversely to the above some studies have also reported that the administration of DHEA did not have any positive effect on mood and well-being. For example, after a 2-week treatment with 50 mg DHEA daily or placebo, 75 elderly men and women

(mean age, 67 years) were exposed to the Trier Social Stress Test (TSST), a commonly used psychological stress test. The TSST induced significant increases in salivary and plasma cortisol. Those women that took DHEA showed ACTH stress responses similar to those of men, but significantly enhanced compared to those of women taking placebos ($p < 0.009$). DHEA treatment did not result in an improvement of subjective well-being (Kudielka et al., 1998).

Depression

Overview

Depression is recognised as a mental illness worldwide and is both included in the Diagnostic and Statistical Manual of Mental Disorders 4th Edition DSM-IV (American Psychiatric Association, 1994) and in the International Classification of Diseases Manual 10th Edition (ICD-10).

Major Depressive Disorder is the most common of the affective disorders. According to the World Health Organisation (WHO), it is one of the most common and debilitating disorders worldwide and the most common psychiatric condition with a lifetime prevalence of 16.2% in the United States (Kessler et al., 2007). Diagnostic criteria for Major Depressive Disorder using DSM-IV and ICD-10 are included in the Appendix.

Treatment Resistant Unipolar Depression

Depression has an episodic course and a tendency to relapse. Although resolution of depressive symptoms is the norm, 10 to 15% of patients suffer with intractable symptoms that persist despite medication adherence and adequate therapeutic trials (Nemeroff, 2007). Although there is 90% recovery of a depressive episode the chances of recurrence are as high as 95% over 16 years (Angst and Wicki, 1992). According to DSM-IV, to be considered separate episodes, there must be an interval of at least 2 consecutive months in which the criteria for a Major Depressive Episode are not met. In the STAR*D trial the overall cumulative remission following 4 acute treatment steps was 67% (Rush et al., 2006) confirming that in real world practice, treatment resistance is common.

Its ubiquitous nature and the associated functional impairment make major depression a major cause of disease burden around the world (Murray and Lopez, 1997; Murray et al., 2000); due to its longer duration and severity, TRD significantly contributes to the above burden.

Measurement of biological markers relevant to the aetiology of treatment resistance has rarely been undertaken. Research into the HPA axis suggests that treatment resistance is associated with glucocorticoid resistance (Bauer et al., 2002). Glucocorticoid resistance is ineffective action of glucocorticoid hormones predominantly cortisol on target tissues. This could lead to immune activation. Equally inflammation could stimulate HPA axis activity via both a direct action of cytokines on the brain and by inducing glucocorticoid resistance (Zunszain et al., 2011). The biological profile of depression that then tends to present with multiple lifetime episodes and treatment resistance could

be different to that of uncomplicated depressive disorder. These abnormalities could either precede the cascade of episodes as well as their severity or could form their unique/different neurophysiological mechanism.

In order to stage Treatment Resistance in depression various staging methods have been used such as the Massachusetts General Hospital staging method MGH-S, (Fava et al., 2003), the European Staging Method and more recently the Maudsley Staging Method (MSM), a predictor of short and long term persistence of symptoms (Fekadu et al., 2009b). A meta-analysis of clinical trials reported rates of partial response in 12–15%, and non-response in 19–34% among antidepressant-treated patients (Fava and Davidson, 1996) and authors from a more recent study concluded that the long-term outcome of depression does not appear to have changed in the last 20 years (Eaton et al., 2007).

Some studies suggest that the TRD group may have an overrepresentation of patients with a Bipolar Disorder and that this contributes to treatment resistance due to incorrect treatment strategies. Sharma and colleagues examined the above hypothesis and concluded that following administration of the SCID-I questionnaire in 69 patients initially diagnosed as TRD, 35% of these were re-diagnosed as suffering with Bipolar Disorder mostly of Type II (Sharma et al., 2005) and to a much lesser extent having a bipolar diathesis or belonging to a “soft” bipolar spectrum. Although in this project subjects who had a bipolar diathesis were not excluded from the unipolar group, most of the patients included were on multiple antidepressants, which would have excluded the majority of iatrogenic cases of Bipolar Disorder. Furthermore a lot of the patients were

unresponsive to mood stabilisers including lithium therapy, the gold standard for Bipolar Disorder.

If there are indeed endocrine differences between Unipolar and Bipolar Depression, this could in fact shed some light as to whether treatment refractory groups may in fact have higher rates of a broadly defined Bipolar Disorder (Angst et al., 2007), as one of the main problem in diagnosis and correct classification is based on the fact that in psychiatry as opposed to various other medical fields, classification of disorders exist based exclusively on subjective symptom description which introduces observer bias. A classification based on neurobiological findings will be much more robust and less prone to diagnostic errors.

Treatment Resistant Bipolar Depression

Bipolar Depression consists part of the longitudinal course of Bipolar Disorder. Bipolar Disorder is the 6th leading cause of disability worldwide (Murray et al., 2000), albeit with a lifetime prevalence of just under 4% (Kessler et al., 2007). Its annual costs exceed those of diabetes and recurrent Unipolar Depressive Disorder (Simon et al., 1999). Although the episodes of Bipolar Depression are similar to those of Unipolar Depression a diagnosis of Bipolar Disorder is given if a person has suffered with at least one hypomanic or manic episode (respectively characterised as Bipolar Type I and Type II). The definition of Bipolar I and Bipolar II according to DSM-IV is the following: to be diagnosed as Bipolar I, an individual must have at least one manic or mixed episode (lasting for at least a week) within his or her lifetime. A depressive episode is not required in order to warrant a diagnosis of Bipolar I, although most people usually have multiple depressive episodes. In order to receive a diagnosis of Bipolar II

Disorder, one must have had at least one hypomanic episode and at least one depressive episode within his or her lifetime. The patient must have never had a manic episode.

Bipolar Depression represents a larger illness burden than the opposite pole within Bipolar Disorder as it comprises in most patients the greatest majority of episodes (approximately 80%). It is the leading cause of impairment and death among patients with Bipolar Disorder (Perlis et al., 2006). Research specifically in Bipolar Depression is lacking compared to Unipolar Depression partly due to the rarity of the condition (compared to Unipolar Depression).

TRBD constitutes a considerable proportion of the overall burden of the condition. As mentioned research is lacking and although studies tend to cluster the two types of depression together there is some evidence that shows that Bipolar Depression is clinically distinct characterised by more atypical features (Bowden, 2005; Mitchell et al., 2001) and agitation within episodes.

Refractory Bipolar Depression has been defined as a depression without remission despite two adequate trials of standard classes of antidepressant agents (at least 6 weeks each and adequately dosed) with or without augmentation (Sachs, 1996). These were also the criteria used in a recent large multicentre European Study (Mendlewicz et al., 2010).

One of the main reasons that the above criteria were used to define TRBD was that recruitment of subjects started in 2006 at which time no medication had gained a license and been approved by the Food and Drug Administration (FDA) and the European

Medicines Agency (EMA) for the treatment of Bipolar Depression. Therefore in the main studies to date, the diagnosis of TRBD has been based on the same criteria as for those of TRUD (Sachs, 1996). This means that the definition has not been based either on the use of quetiapine or olanzapine/fluoxetine, the only medications that are licensed for Bipolar Depression and TRD by the FDA, or on mood stabilisers.

A paper by Young and MacPherson highlights that Bipolar Disorder includes a number of subtypes including Bipolar I Disorder, Bipolar II Disorder and bipolar spectrum (Young and MacPherson, 2009). The differentiation between these subtypes essentially rests on the severity of the pathological elevations of mood, with mania being unique to Bipolar I Disorder and hypomania characteristic of Bipolar II Disorder and sub-clinical hypomania occurring in bipolar spectrum. The inclusion criteria and the diagnostic thresholds for Bipolar Disorder have been suggested by prospective cohort studies to be too restrictive to adequately recognise bipolar spectrum in the general population, notably more in young adults when the disorder is in its early stages. More importantly in the BRIDGE study it has been suggested that by applying both DSM-IV criteria and previously described bipolarity specifier criteria approximately 47% of patients with past mood episodes scored positive on these (Angst et al., 2011).

A recent paper by Li and colleagues reported an association in antidepressant response history and subsequent change in diagnosis from depression to Bipolar Disorder (Li et al., 2012). Using a nationwide database in Taiwan, patients with TRD had a subsequent change of diagnosis to Bipolar Disorder (25.6%-26.6%). The main finding was that participants with easy to treat depression were less likely to convert to Bipolar Disorder, whereas those that were more difficult to treat were more likely to convert to Bipolar

Disorder. According to the authors, this supports the notion that there is a link between depression that is resistant to treatment and Bipolar Disorder especially Bipolar II. According to the above study it may also be that certain antidepressants such as TCAs or SNRIs could induce switching however the use of these medications did not fully explain the switching.

Issues in relation to the definition of TRBD are further described in a recent paper by Vieta and colleagues (Vieta et al., 2011). Regarding our study, definition of TRBD is described in Chapter 2.

LITERATURE REVIEW

In this section the existing literature on HPA axis disturbance (cortisol, DHEA and their ratio) is reviewed.

Cortisol

Cortisol and Unipolar Depression – overview

Abnormal cortisol in depression has been shown with the Dexamethasone Suppression Test (DST). The initial hypothesis is that patients with depression have an abnormal HPA axis, as also shown in conditions like Cushing's and Addison's diseases. Therefore the HPA axis was assessed by using dexamethasone, a synthetic steroid which in controls should produce a decrease in plasma cortisol levels, by lowering the HPA production of CRH by negative feedback and thereby reducing ACTH and the adrenal production of cortisol.

In a study by Carroll and colleagues 438 subjects underwent an overnight DST using 1 mg and 2 mgs of dexamethasone. According to this study, abnormal plasma cortisol concentrations within 24 hours after DST administration occurred almost exclusively in patients with melancholic depression. Two blood samples obtained at 4 and 11 pm following dexamethasone administration detected 98% of the abnormal test results. Thus, this version of the test was able to identify patients with a sensitivity of 67% and a specificity of 96% (Carroll et al., 1981).

With variations that depend on specific assay times, as well as time of plasma collection and amount of dexamethasone administered, plasma levels of cortisol post suppression

should be, within 17 hours, below 690 nmol/L. However, although initial studies reported the DST test to have a moderate sensitivity (40% to 50%) and a high specificity (90% to 95%) for the diagnosis of depression (Carroll et al., 1981; Baldessarini and Arana, 1985), these data were not supported by subsequent studies (Stokes and Sikes, 1988). Thus, the use of DST in depression has decreased in recent years.

Gold and Chrousos, (1985) and Holsboer and Benkert, (1985) were among the first to report hypercortisolemia in depression. Although the authors related the above finding to a blunted plasma ACTH response to ovine CRH and hypercortisolemia of central origin (Gold and Chrousos, 1985), peripheral abnormalities are also well replicated in depression. Even before then, hypercortisolemia has been described in psychotic depression in a study by Sachar and colleagues described below (Sachar et al., 1973).

However, a recent meta-analysis (Knorr et al., 2010) casts some doubt on the presence of hypercortisolemia in depression. In this review 20 case control studies were included, with a total of 1354 depressed patients or offspring at risk and 1052 controls.

Measurement of morning cortisol was made in saliva. No firm evidence was drawn regarding salivary cortisol between groups, however, due to large heterogeneity for the morning analysis and possible presence of bias; thus, the results should be interpreted with caution. In addition, only 13 of these studies reported HAM-D or similar depressive scores, whereas the rest did not; thus the degree of severity of depression is unclear in a substantial number of these studies. Furthermore, inclusion criteria did not differentiate between different forms of depression which are known to exhibit different biological profiles. Therefore patients with both atypical depression, a depression

subtype that exhibits hypo rather than hypercortisolemia (Juruena et al., 2007; Gold et al., 2002), and Bipolar Disorder (including testing during manic phases) are also included in the review.

Some other studies measuring cortisol in various forms of depression are hereby mentioned:

Unipolar Depression (Blood/plasma)

Cortisol was measured in 18 patients with Major Depressive Disorder of the endogenous subtype (8 unipolars and 10 bipolars) who were submitted to blood sampling at 15 min interval for 24 hrs. Hypercortisolemia was evident in the depressed patients throughout the 24 h span. These abnormalities were more pronounced and more consistent in patients with Unipolar rather than Bipolar Depression (Linkowski et al., 1988).

Posener and colleagues also measured cortisol collected in blood at hourly intervals over a 24-h period in 15 medication-free men with Major Depressive Disorder and 15 healthy controls. Depressed men exhibited raised cortisol compared to controls, a finding possibly suggesting a loss of regulatory control over cortisol secretion and an increased cortisol feedback on the pituitary (Posener et al., 2004). To test the latter hypothesis the authors conducted a study where 29 patients with major depression and 25 healthy controls were randomly assigned to administration of 15 mg of cortisol or placebo infused over 2 hours beginning at 0700 hrs. No differences were found between patients and controls leading to a conclusion of a normal negative feedback mechanism (Posener et al., 2001).

Cortisol activity following antidepressant treatment has also been measured in plasma. Schule and colleagues measured morning plasma cortisol in 23 (4 men and 19 women) patients with depression (only melancholic cases of depression were included and atypical, psychotic and Bipolar Depression was excluded), who received a 5-week treatment with 45 mg of mirtazapine, but no other psychotropic drugs. Patients have never been pretreated with mood stabilisers or neuroleptics, had a mean HAM-D 21 Item score of 18 and had suffered with a mean of 5 previous depressive episodes. The duration of the current episode was 153 days. Plasma samples were taken before mirtazapine therapy and every week thereafter at 0800 hrs. Following this period of treatment cortisol levels decreased. DHEA-S levels, also evaluated in this study are described later in the chapter (Schule et al., 2009).

Unipolar Psychotic Depression (plasma)

One of the first and most pioneering studies measuring cortisol in the field of psychotic depression reported a disrupted 24-hour pattern of plasma cortisol secretion in 6 subjects compared to 8 controls. Total secretion of cortisol remained elevated even during the late evening and morning hours when secretion of cortisol normally remains minimal (Sachar et al., 1973). As noted the number of subjects in the study is very small.

Not all studies however agree regarding plasma cortisol and psychotic depression. Cortisol was measured during a 24-hr period in 11 subjects with psychotic depression, 38 subjects with non psychotic depression and 11 controls recruited from outpatient services (Posener et al., 2000). Diagnosis was based on DSM-III criteria and severity of depression was measured by the HAM-D 21 Item Rating scale (cut off score of 21).

Subjects were medication free (no psychiatric medication in the 2 weeks before and during the study, no fluoxetine in the previous 6 weeks and no depot neuroleptics in the previous 3 months). With regards to chronicity and severity, psychotic depressed patients suffered with more chronic and severe depression. Depressive disorder was not however clearly defined in the study. Subjects attended a research lab where an intravenous line was inserted and blood was drawn every hour until 0600 hrs the following day. Cortisol indices including mean cortisol did not differ between patients and controls although the cortisol amplitude (in the study defined as maximum minus minimum values) was lower ($p=0.046$), with a post hoc test revealing a lower amplitude in non psychotic major depressed patients compared to controls. This finding remained after excluding 6 patients with a concomitant PTSD diagnosis. Psychotic depressed patients did not differ significantly from either of the other groups.

Unipolar Psychotic Depression (urine)

Keller and colleagues compared free urinary cortisol during a 24-hour period in 37 subjects suffering with psychotic major depression, 32 subjects with non psychotic major depression and controls. Depressed patients had a minimum score of 21 on the Hamilton Depression Rating Scale (Hamilton, 1960). Subjects with psychotic depression had a minimum score of 5 on the BPRS (Overall et al., 1961). The main outcome in the study was that cortisol levels were higher in psychotic depression between 1800 hrs and 0100 hrs compared to non psychotic patients and controls although no differences were observed between groups when cortisol was measured in the morning hours (Keller et al., 2006). This enhancement of cortisol activity during quiescent hours suggests a defect in the circadian timing system and HPA axis and potentially an imbalance of corticoid receptors.

Unipolar Depression (saliva)

Studies have reported unaltered levels of cortisol between patients and controls. Assies and colleagues examined both salivary cortisol and DHEA-S levels in 13 medicated, unipolar, non-psychotic depressed patients, with a mean HAM-D 17 Item score above 15 and in 13 healthy volunteers. All patients used antidepressants and 5 also used benzodiazepines. Depressive disorder was not defined in the study. Diurnal declines in cortisol and DHEA-S levels were found in both depressed and control groups. In patients compared with controls, DHEA-S was significantly elevated, in conjunction with normal cortisol levels (Assies et al., 2004).

Unipolar Depression in children and adolescents (saliva)

Goodyer and colleagues measured cortisol in 82 children and adolescents (8-16 years). Salivary hormone levels at 0800, 1200 and 2000 hrs, were collected over a 48 hour period. Cortisol was raised in those subjects suffering with depression (Goodyer et al., 1998). Goodyer did not assess cortisol in isolation but mostly concentrated on measuring the ratio of Cortisol/DHEA and his studies are described later on in this chapter.

Increased salivary cortisol at 0800 hrs was associated with prediction of chronic depression at 36 weeks and 72 weeks follow up (Goodyer et al., 2001).

Cortisol Awakening Response Unipolar Depression (saliva)

The CAR and its relation to depression has often been evaluated giving inconsistent results which is possibly related to sample heterogeneity.

The salivary CAR was measured in a community based sample of 20 unmedicated depressed patients and 40 controls. Subjects were recruited from primary care and their mean age was 43 years old. Use of oral contraceptives was not an exclusion in this study (3 patients and 3 controls). Depression was not clearly defined in the study. Apart from this subjects were medication free for a minimum of 4 weeks and had a mean 17 Item HAM-D score of 20. Subjects were given clear instructions to collect saliva at home at 15-minutes intervals for the first hour following awakening. There was a trend towards AUC of salivary cortisol secretion measured by the trapezoidal method being higher in patients compared to controls ($p=0.059$). No correlation was found with the HAM-D score. Although secretion of cortisol over the first 60 minutes was higher by 25% in patients compared to controls, absolute levels of cortisol were comparable at the 60 minute time point (Bhagwagar et al., 2005).

The CAR was also studied in patients with depression and comorbid coronary artery disease, as cortisol is linked to atherogenesis and vascular inflammation that is often involved in coronary heart disease (Brotman et al., 2005). Subjects suffering with depressive disorder have an adverse prognosis following acute coronary syndrome although it is not clear whether this is related to cortisol. In a study by Bhattacharyya and colleagues, 88 out of 144 subjects eligible to take part due to being referred with chest pain were recruited from 3 primary hospitals in London as possible sufferers of coronary artery disease. The BDI (Beck Depression Inventory) was applied and subjects were consequently split into those who scored above and below 10. Salivettes were used to collect saliva on day 1 between 0900 and 1000 hrs and then at 1100 hrs, 1600 hrs, 1900 hrs and prior to sleeping. On day 2 they were instructed to collect saliva at 0, 15 and 30 minutes following awakening. Actigraphy was used to measure patients'

activity. Analysis was undertaken between patients with and without depression and with and without heart disease. The CAR (measured by the trapezoidal method) was higher in patients that suffered with coronary artery disease compared to those that did not ($p=0.04$); it was not however related to depression (Bhattacharyya et al., 2008).

Cortisol Awakening Response in adolescents (saliva)

To examine the hypothesis that a higher CAR may lead to prediction of depression at 1 year follow up, Adam and colleagues measured salivary free cortisol at 0 and 40 minutes following awakening in 627 adolescents using the drooling method. The Structured Clinical Interview for DSM-IV (SCID) (First et al., 2002) was used for diagnostic purposes. 24% of the sample was diagnosed as suffering from a depressive episode at the time of the first assessment. Although there was no association between past episodes of depression and the size of the CAR at baseline, prediction of depression at follow up measured by SCID and Life Charts was possible by the magnitude of the CAR, but not by other cortisol parameters such as diurnal cortisol slope and average cortisol during the day, a finding that gives important significance to the CAR compared to other ways of measuring cortisol activity (Adam et al., 2010).

Vreeburg and colleagues measured the CAR in 579 persons with remitted MDD, 701 subjects with current MDD and 308 controls. The CAR was measured in these participants over 4 time points (0, 30, 45, 60 min). Both the AUCi and AUCg were calculated. The CAR AUCg was higher in patients with depression compared to controls (AUCg $p=0.001$, AUCi $p=0.28$). Patients with comorbid GAD had an even higher CAR. Furthermore the CAR remained higher in patients with remitted MDD

(AUC_g $p=0.02$, AUC_i $p=0.04$) possibly representing a state rather than trait marker of the condition.

The DST test was also measured. Evening cortisol was higher in patients at 10 pm. Rates of non suppression to the DST were not different among depressed patients compared to controls possibly related to the fact that dysregulation of the HPA axis sufficient to confer resistance to dexamethasone suppression is most confined to severe psychotic depression (Vreeburg et al., 2009).

Cortisol Awakening Response in Remitted Unipolar Depression (saliva)

To assess whether the CAR continues to be raised following remission in depression, in line with other neurobiological abnormalities that persist following recovery (Bhagwagar et al., 2008), a number of studies have been designed. Once again results have been inconsistent. In order to test the above hypothesis salivary cortisol collected in salivettes was measured every 15 minute during the hour following awakening in 31 medication free recovered depressed patients and 31 matched controls. All patients were in remission (DSM-IV) and had been in remission for at least 6 months. However, on the Structured Clinical Interview for DSM-IV disorders (SCID), although 28 patients met criteria for recurrent depressive disorder 3 met criteria for Bipolar Disorder (Type II). HAM-D 17 Item was applied to measure remission (mean score was 2). Although patients had achieved remission hormonal abnormalities persisted as shown by a higher CAR compared to controls ($p<0.02$) (Bhagwagar et al., 2003).

A more recent study measured the CAR in patients having remitted from at least 3 previous depressive episodes. 11 men and 27 women were screened for past depressive

episodes by an experienced clinician with the Structured Clinical Interview for DSM-IV (SCID-First et al., 1996). Depressive symptoms were assessed with the Montgomery and Asberg Depression Rating Scale (MADRS- Montgomery and Asberg, 1979). All patients were in remission and off medication for at least 3 months. Saliva was collected at home using salivettes. CAR as measured by the AUC was found to be 51% higher compared to controls after adjusting for variables such as weekends versus weekdays, wake-up time and sleep duration (Aubry et al., 2010).

Related to the recovery process, there has been an evaluation of the effects of different categories of antidepressants on the cortisol response to awakening. Harmer and colleagues studied the short term effect (6 days of treatment) of 20 mgs of citalopram, a Selective Serotonin Reuptake Inhibitor (SSRI) and 8 mg reboxetine, a Noradrenergic Reuptake Inhibitor on diurnal salivary cortisol secretion in a placebo-controlled double blind study using salivettes. Although citalopram led to an increase in salivary cortisol production in the morning following awakening, the effect of reboxetine was comparable to placebo (Harmer et al., 2003).

Cortisol and the Cortisol Awakening Response in Treatment Resistant Unipolar Depression

Although cortisol has been extensively measured in depression even in patients experiencing multiple depressive episodes, the effect of cortisol or the CAR has never been clearly evaluated in TRD. This is partly related to the poor definition of TRD. Another factor that may have contributed to this is that subjects with resistant depression are extremely difficult to recruit in research studies. Hypercortisolemic

depressed patients included in numerous studies as mentioned on the other hand have often experienced numerous depressive episodes.

Cortisol and childhood abuse in Unipolar Depression

Increased cortisol reactivity has been described in subjects with childhood abuse (Heim et al., 2000), with a similar effect of social anxiety on cortisol following a stress test (Elzinga et al., 2010). The effect of abuse has not been evaluated in TRUD.

Cortisol and Bipolar Depression (blood/plasma) including TRBD.

Rasgon and colleagues measured plasma cortisol in 6 bipolar depressed women with a score of ≥ 15 on the 21-item Hamilton Rating Scale and compared this to 5 controls. Psychotic features and suicidal risk were exclusion criteria for the study. Subjects with bipolar disorder had been unresponsive to at least 6 weeks of antidepressant treatment. All patients had received the same antidepressant during the 6 weeks prior to study entry, with no changes in dose during the 3 weeks prior the study entry or during study treatment. The protocol included overnight blood sampling. Subjects were admitted to the lab suite where an intravenous line was inserted at 1900 hrs. Blood was drawn every 15 minutes from 2100 to 0900 hrs the following morning. The same procedure followed at the end of the 7 week trial. During the trial females received T4 (levothyroxine) once daily at a dose of 100 µg/day for the first week, 200 µg/day for the second week and 300 µg/day for weeks 3-7 (Rasgon et al., 2007). This was an 'add-on' treatment although all the patients were medically stable, euthyroid and almost all were tested during the luteal phase of their menstrual cycle both during the beginning and end of the trial. Studies suggest that patients with refractory depression could benefit from the use of T3 (triiodothyronine) as an 'add on' treatment, which may augment the therapeutic

response to antidepressants and T4, which can ameliorate depressive symptomatology and help stabilise the long-term course of illness in bipolar and unipolar patients, especially in women refractory to standard medications (Bauer et al., 2002).

Compared to controls, bipolar patients had significantly lower maximum cortisol levels and showed lower amplitude (in this study defined as maximum-mean levels) of cortisol and trends were noted for overall mean ($p=0.067$) and variation (maximum-minimum levels) ($p=0.06$).

Following treatment with T4, cortisol levels decreased in bipolar patients however no significant correlation was found. If TRBD is defined as failure to respond to one treatment trial as opposed to two, then this is the only previous study to measure cortisol in this group of patients.

In another study –which in contrast reported cortisol hypersecretion in Bipolar Disorder – 18 subjects were included, but only 5 were in the depressive phase of their illness. Depression was rated via the HAM-D Rating Scale; the Young Mania Rating Scale (YMRS) was also applied as there was also a participation of 8 patients during the euthymic and 5 during the manic phase of their illness. 24-hr blood sampling was performed after installing an intravenous catheter at 0700 hrs: blood collection initiated at 0800 hrs and continued at hourly intervals for 24 hours. ANOVA for the Area Under the Cortisol Curve showed higher cortisol release in patients during the depressive phase ($p=0.03$) (Cervantes et al., 2001).

Another study by Linkowski and colleagues included 8 subjects with unipolar depression and 10 bipolar depressed patients. As mentioned above, in this study plasma cortisol was measured each 15 minutes for 24-hr. Hypercortisolism was evident during this 24 hr span. A sub-analysis between groups revealed more pronounced abnormalities in Unipolar compared to Bipolar Depression (Linkowski et al., 1988). Finally cortisol was increased following administration of ipsapirone, a 5-HT_{1a} receptor agonist, in 8 patients with Bipolar Depression compared to controls (Shiah et al., 1998).

Related to the above a recent study by Ellenbogen and colleagues reported higher evening cortisol in the offspring of parents with Bipolar Disorder during 2 weeks of daily sampling (Ellenbogen et al., 2010). 24 offspring that had a positive family history of Bipolar Disorder (at least one parent with Bipolar Disorder) and 22 offspring without a history of Bipolar Disorder recruited from the same geographical regions were included in the study. High risk individuals whose parent suffer with an affective disorder have a preponderance for an affective disorder as 30-50% will develop one during their lifetime. Following a 2 week sampling, cortisol was found to be higher in the afternoon in the high risk offspring. Cortisol was collected from 1300 hrs to 1500 hrs and at 2000 hrs to bedtime.

Cortisol Awakening Response Bipolar Depression (saliva)

The CAR has been evaluated in Bipolar Depression albeit in a small number of studies. Ellenbogen and colleagues compared in a pilot study salivary cortisol levels over 2 days in 10 adolescents whose parents suffered with Bipolar Disorder and another 10 whose parents were illness free. The adolescents did not themselves suffer with any form of mental illness. The offspring whose parents suffered with Bipolar Disorder exhibited

higher morning and afternoon cortisol levels. Cortisol continued to remain raised in the afternoon after controlling for age (Ellenbogen et al., 2004).

The same authors sought to evaluate the CAR and basal cortisol levels over 2 days in a larger sample of high-risk and low-risk offspring (defined as above). Saliva samples were collected from 29 high risk and 29 low risk adolescents whose mean age was 15. Diagnosis was via the DSM-III-R and from an examination of psychiatric records. Cortisol was measured via salivettes and the response to awakening was evaluated at 0, 30 and 60 minutes. AUC was found to be higher in high risk compared to low risk offspring ($p < 0.05$). Subjects then underwent either a standard version of the "Trier Social Stress Test" or a child adaptation if less than 15 years and saliva was again collected at 10, 20, 30 and 45 minutes following the test. High-risk offspring had higher daytime levels of cortisol than low-risk offspring. This was higher for females irrespective of their risk status compared to males. The cortisol response to the laboratory psychosocial stressor was not different between the 2 groups. The authors concluded that children of parents with Bipolar Disorder have an increased daytime basal HPA functioning but show a normal reactivity to psychosocial stress (Ellenbogen et al., 2006).

In a more recent study the same authors hypothesised that parenting style towards the offspring of Bipolar Disorder patients confers adversity and may influence the HPA axis. They found that low levels of structure in parenting are predictive of raised cortisol response following awakening (Ellenbogen et al 2010). The CAR was also evaluated over 2 days in another recent study by the same author (Ellenbogen et al., 2010). 24 high risk offspring as defined above and 22 offspring without a history of Bipolar

Disorder were included in the study. Cortisol was measured on awakening and after 30 and 60 minutes using salivettes. Risk status was not predictive of overall cortisol levels but this negative finding was attributed to the study being underpowered.

Cortisol Awakening Response in Remitted Bipolar Depression (saliva)

Following a body of evidence that suggests that cortisol abnormalities persist after recovery, a pilot study was conducted by Deshauer and colleagues who measured cortisol following awakening in 18 clinically stable lithium responsive patients with a history of Bipolar Disorder of either Type I or II. Saliva was collected each 15 minutes following awakening for the duration of an hour. Although clinically stable and on lithium prophylaxis patients with Bipolar Disorder showed a significantly enhanced salivary cortisol response to awakening compared to controls ($p < 0.03$) (Deshauer et al., 2003).

The same authors consequently conducted a larger study in an attempt to replicate their previous finding. Intensive cortisol sampling was conducted (6 samples per day for 3 test days, on 3 consecutive weekends) on 15 patients suffering with Type I and II Bipolar Depression, 28 unrelated high risk offspring of bipolar parents and matched controls. Participation was restricted to cases in complete sustained remission. There was no statistically significant difference in cortisol secretion at any sampling time between remitted bipolar patients, remitted offspring of bipolar parents, and normal controls. The CAR did not differ between patients and controls. Therefore in this case complete sustained clinical remission was associated with normal salivary cortisol levels throughout the day and a personal or family history of bipolar disorder *per se* did

not appear to confer added risk for increased salivary cortisol secretion during this (Deshauer et al., 2006).

Cortisol and the Cortisol Awakening Response in Treatment Resistant Bipolar Depression

Other than the small study described earlier (Rasgon et al., 2007) the effect of cortisol on TRBD has never been evaluated. Reasons why there have been no previous studies in TRBD include the problems in always accurately differentiating Unipolar and Bipolar Depression, given the similarity in cross-sectional presentation. Furthermore, there has yet to be established a consensus on the definition of TRBD amongst clinicians, which makes the design of a study attempting to examine a homogeneous clinical sample difficult. It is also of note that to date there have been no clear studies that have reported the prevalence of the condition.

Cortisol and abuse in (Treatment Resistant) Bipolar Depression

This has not been evaluated.

DHEA/DHEA-S

DHEA/DHEA-S and Unipolar Depression (plasma)

Studies measuring DHEA in plasma have provided inconsistent results, this sometimes depending on whether DHEA or DHEA-S was measured. 699 postmenopausal women were followed up in a cohort study and screened for depression. Single DHEA-S measures were obtained between 0730 and 1100 hrs and DHEA-S was found to be lower in depressed women compared to controls. In a subsample of 31 depressed

women and 93 controls DHEA-S but not DHEA was found to be inversely correlated with depression (Barrett-Connor et al., 1999).

Scott and colleagues also reported lower DHEA-S in 15 subjects (8 females and 7 males), suffering with depression recruited from a Chronic Fatigue clinic (CFS). The study also reported DHEA/DHEA-S results in CFS patients; however these findings will not be reported here. All depressed subjects had a DSM III-R diagnosis of major depression and were also assessed by a structured interview (SCID). Severity of depression was assessed by the HAM-D score, the mean score of patients being 24. Subjects with an Axis II diagnosis were not included in the study. None of the participants was on any medication known to affect the HPA axis in the 4 weeks preceding the trial. Plasma samples were taken for cortisol and DHEA/DHEA-S between 1200 and 1400 hrs. Cortisol levels did not differ between groups. DHEA/DHEA-S levels were found to be lower in depressed patients than controls. DHEA levels were not different between depressed subjects and controls (Scott et al., 1999). Furthermore the Cortisol/DHEA and Cortisol/DHEA-S ratios were higher in depressed patients compared to controls.

Buckwalter and colleagues tested 19 pregnant women during the last 2 months of pregnancy and first two of delivery. Participants, all of whom had uncomplicated pregnancies were recruited via a private practice in an inner city area. Mood was evaluated via the BDI (Beck Depression Inventory), POMS (Profile of Mood States) and SCL (Symptom Check List-90). To assess correlations between mood and cognition and neuroendocrine measures DHEA and cortisol were measured in blood 20 days pre and post delivery. Although subjects were not overtly depressed (mean BDI score in

pregnancy was 10 and following delivery was 8), they exhibited some symptoms of depression as shown by a mean SCL score of 58. During pregnancy, higher levels of DHEA were associated with better mood as shown by a correlation with lower BDI scores and SCL, but after pregnancy DHEA only correlated with lower SCL. Following pregnancy DHEA and cortisol were correlated with better cognitive performance (Buckwalter et al., 1999).

Serum DHEA-S was measured in 28 medication free outpatients (9 males and 19 females) with a mean age of 33 that met DSM-IV criteria for Major Depressive Disorder. Subjects included in the study attended the outpatient clinic. A cut off score of 17 on the HAM-D 17 Item Rating Scale was used. Anxiety and depression were further assessed using the HADS questionnaire (The Hospital Anxiety and Depression Scale), a self rated measure of anxiety and depression. DHEA-S levels measured in the morning between 0900-1100 hrs correlated with the depressive subscale of HADS but not with the HAM-D (Hsiao, 2006).

In contrast to the above findings relating depression to low DHEA levels, there have been a number of studies that report increased rather than decreased levels of DHEA in depression. Heuser and colleagues studied 24-h DHEA plasma concentrations in 26 depressed patients (21 HAM-D Item above 18) and 33 healthy controls. Blood specimens were withdrawn each 30 minutes for 24 hours via an indwelling catheter. Mean cortisol was increased in depressed patients compared to controls. Mean DHEA was also increased in the patient group compared to controls both in younger and elderly subgroups (Heuser et al., 1998).

Other studies have shown no change in DHEA levels. Erdinler and colleagues studied plasma levels of DHEA-S in 74 elderly women 39% of whom (34 females) were suffering with depression and controls. Depression in patients was assessed via the Geriatric Depression Scale (GDS) (Yesavage et al., 1982). Mean GDS score was 18. No information is given related to the use of psychotropic medication. Subjects who had a clinical acute or chronic illness were excluded from the study. Depression was evaluated using the DSM-IV diagnostic criteria (Erdinler et al., 2004). Hsu and colleagues reached a similar conclusion whilst assessing 80 patients undergoing hemodialysis. Depression is a common problem in subjects undergoing hemodialysis; the prevalence of depression of the above sample was 37.5%. Therefore 30 patients were included in the sample. Depression was evaluated using the HADS, a 14-Item self administered questionnaire containing 7 items related to depression and 7 items related to anxiety. No information is given on medication use by the subjects. Blood samples were collected from the patients between 0730 and 1100 hrs, after the patients had a 12-hr fast. DHEA-S was not found to be different between patients with and without depression, thus suggesting a lack of association between depression and DHEA-S in hemodialysis subjects (Hsu et al., 2009).

DHEA/DHEA-S and treatment response

DHEA-S has also been evaluated when measuring treatment response. Plasma DHEA-S (but not DHEA or cortisol) obtained before and after the first and sixth ECT session in 17 hospitalised patients suffering with schizophrenia, schizoaffective disorder and depression, as defined by DSM-IV, were found to be elevated following treatment response. Patients with psychotic depression were found to have higher DHEA levels

compared to controls. Of interest is that non-elevated basal DHEA-S levels were associated with clinical response to ECT (Maayan et al., 2004).

Plasma DHEA has been measured following antidepressant treatment. Hsiao and colleagues measured plasma morning DHEA in 34 patients (9 males and 25 females) pre and post treatment with 75 mg of Venlafaxine XL for a 6 week period. The Hamilton Rating Scale for Depression 17 Item (cut off score 17) and the MINI, a short structured interview for DSM-IV (Sheehan et al., 1998) were applied to all subjects. Subjects had a mean duration of illness episode of 6 months. 44% of the subjects achieved remission by the end of the study as defined by a HAM-D 17 Item score of less than 7. DHEA was drawn from all subjects from 0900-1100 hrs. It was found to decrease following remission ($p=0.017$) which correlated with a decrease in the HAM-D score (Hsiao, 2006).

Similar results were presented by Fabian and colleagues who also identified a reduction of DHEA in a sample of elderly depressed patients who remitted or failed to remit following 12 weeks of treatment with either nortriptyline or paroxetine. 60 elderly patients were enrolled in this double blind trial. All subjects enrolled met DSM-IV criteria for depressive disorder and a cut off 17 Item Hamilton Score of 15. All medication was discontinued for the duration of the trial except lorazepam for anxiety or agitation. Bloods were collected at baseline and after 12 weeks between 0700 and 0900 hrs.

By the end of the 12week trial 73% of the sample, 44 patients were in remission (HAM-D 17 Item less than 10 over 3 consecutive weeks). DHEA levels were lower in remitters

at week 12 compared to week 0 ($p=0.002$). Although cortisol levels were lower in both remitters and non at week 12 these results were not statistically significant. The ratio of Cortisol/DHEA was not found to be different between patients and controls nor did it change significantly from week 0 to week 12 (Fabian et al., 2001).

In a similar study, Schule and colleagues both morning plasma cortisol and DHEA-S were measured in 23 subjects with Major Depressive Disorder (4 males and 19 females) diagnosed by DSM-IV. Depression was further assessed by the HAM-D 21 Item (cut off score ≥ 18). Information on previous depressive episodes or whether these subjects were resistant to treatment is not given. Blood was drawn one hour following awakening at 0800 hrs on days 0, 7, 14, 21, 28, 35 of the experiment. 2 men and 10 women responded to the trial (HAM-D reduction $<50\%$). By week 5, a significant decrease in cortisol and DHEA-S, but not their ratio were apparent in subjects treated with 45 mg of mirtazapine for 5 weeks. DHEA-S correlated in a positive way with the percentage of Hamilton reduction by week 5 (Schule et al., 2009).

Paslakis and colleagues measured morning DHEA-S plasma levels in 70 (48 females and 22 males) patients suffering with non psychotic Major Depressive Disorder (DSM-IV criteria) with a mean HAM-D 21 Item of 18 and 33 matched controls. DHEA-S was measured at baseline and following a 4 week treatment period with either venlafaxine (200 ± 58 mgs) or mirtazapine (50 ± 30 mgs). A decrease in DHEA-S levels was observed in depressed patients who achieved remission (Paslakis et al., 2010).

Takebayashi and colleagues showed that following 4 week of antidepressant treatment with clomipramine plasma DHEA-S and cortisol levels decreased. The depressed

patients showed significantly higher baseline values of plasma DHEA-S and cortisol compared to controls which following the antidepressant treatment returned to normality (Takebayashi et al., 1998).

Romeo and colleagues reported no change in the levels of DHEA measured at 0900 hrs every ten days in 8 male depressed outpatients until they reached clinical remission. These subjects were drug naïve and suffered with their first episode of Major Depressive Disorder with psychotic features according to the DSM-IV. The HAM-D 17 Item was applied with a score decreasing from 20 to 9 from the beginning until the end of the assessment. Fluoxetine was administered at a dose of 20 mg in the morning. Plasma samples were obtained on day 0 at 0900 hrs and on every 10 days of treatment until day 50. In a second study, 11 severely depressed inpatients with a mean HAM-D 21 Item score of 28 were examined. The HAM-D score decreased from 28 to 5 following 55 days of treatment. During the treatment period subjects were on various antidepressants and one was on lithium. Plasma DHEA was obtained at 1600hrs at the start and end of the study. No significant changes after remission were observed with regards to DHEA in either of the treated groups (Romeo et al., 1998).

Therefore although most trials do describe a decline in DHEA/DHEA-S following treatment for depression and successful therapy, conclusions are again not consistent. There are various factors that potentially contribute to this:

First, the studies mentioned above are not homogeneous given that very often the sample that is examined is not clearly defined. Different forms of depression are included within a study such as atypical and Bipolar Depression, which potentially have

a different neuroendocrine profile to that of the typical melancholic depression. Thus, apart from severity of depression which is often clearly defined, there is often no clear definition of depression type and symptomatology. Other factors related to the inconsistency in the results are the use of psychotropic medication, the power of the study and often the lack of a placebo group, comorbidity and other confounders such as gender and age. Collection method and whether plasma, urine or saliva are used is also another factor that may confound results, as is also whether DHEA or DHEA-S is measured. Time of collection and/or whether 24-hr concentrations are used is of paramount importance and affects conclusions in the studies. Another point may be that when assessing DHEA/DHEA-S levels in remitted patients, although most studies use a HAM-D score of less than 10, sometimes a 50% reduction of the initial score is used as a criterion, introducing once again inconsistency.

Given all the above reasons it still has not been possible to establish whether DHEA could be used as a biological marker in Affective Disorders and how valid it could be as such (related to its sensitivity and specificity).

DHEA/DHEA-S and Unipolar Depression (urine)

Tollefson and colleagues demonstrated that the total 24-hr urinary DHEA-S was elevated in depressed patients, but that this declined following treatment with imipramine although it is not clear whether this was related to treatment response (Tollefson et al., 1990).

24-hr urinary studies are not however without problems. It is known that although 24-hr studies provide information on the HPA activity over a large period of time the

compliance in the studies is often poor. Renal conditions can sometimes affect urinary levels. Not always does the whole free product get excreted by the urine, as liver is also known to affect metabolism. Clearance rates could also affect levels of urinary DHEA-S.

DHEA/DHEA-S and Unipolar Depression in children and adolescents (saliva)

Goodyer and colleagues measured salivary DHEA in children and adolescents (8-16 year old) in a number of studies. Initially 82 children and adolescents with major depression, 25 non depressed psychiatric cases and 40 controls collected DHEA/DHEA-S over 2 days at 0800, 1200 and 2000 hrs. Mean values of the 2 days were compared between groups. Evening cortisol hypersecretion and morning DHEA hyposecretion were significantly, and independently, associated with major depression (Goodyer et al., 1996). The above 82 children and adolescents were reassessed at 12 months after their initial presentation. Cortisol, DHEA and their ratio were measured at 0800, 1200, 1600, 2000 and 2400 hrs. None of the single parameters at presentations predicted persistence of depression at 12 months. However higher Cortisol/DHEA ratios at 2000 or 2400 hrs predicted persistent major depression at 12 month follow-up (Goodyer et al., 1998).

To further determine whether endocrine abnormalities predict the onset of Major Depressive Disorder, Goodyer and colleagues measured salivary DHEA at 0800 and 2000 hrs in two subgroups of adolescents in the community who were at high and at low risk for psychopathology at entry and again at 12 months. High risk was defined by the presence of greater than 2 undesirable life events over the last 12 months (danger to self, others, disappointments, loss), current or past marital disharmony/breakdown, high emotionality, 2 or more lifetime exit events or that have personal significance to the adolescent and history of parental psychiatric disorder (Goodyer et al., 2000a). DHEA

hypersecretion at 0800 hrs or at 2000 hrs greater than the 80th percentile of the mean during the initial assessment was associated with subsequent major depression (Goodyer et al., 2000a,b) at 1 year follow up.

The long term effect of salivary DHEA was evaluated in 78 clinically referred subjects suffering with depression at 36 weeks and 47 depressed subjects at 72 weeks. Variations in DHEA levels were not associated with chronicity (Goodyer et al., 2001).

Assies and colleagues examined salivary morning and evening levels of cortisol and DHEA-S in 13 patients suffering with non psychotic depression. DSM-IV criteria were applied for the study and a cut off score of ≥ 15 at the HAM-D 17 Item was required for inclusion. All patients were on antidepressants, predominantly SSRIs and 3 females were using oral contraceptives. Information on number of antidepressant treatments and previous depressive episodes is not included in the study. Saliva samples were obtained at 0800 and 2200 hrs although the specific method of collection is not stated in the study. There were no differences in mean cortisol levels or the diurnal slope between patients and controls. On the other hand DHEA-S was higher in the depressed group compared to controls (Assies et al., 2004).

There have been also studies that have shown a negative correlation between DHEA and depression as in a study by Michael and colleagues, in which salivary cortisol and DHEA were measured over 4 days at 0800 hrs and 2000 hrs. 44 (12 males and 32 females) subjects suffering with depression were recruited from the inpatient and outpatient services. Subjects were free from any psychotic phenomena. DSM-IV was used for diagnostic purposes. Hormonal measures were compared to 35 remitted

patients (14 males and 21 females) who were also included in the study and 41 controls. Mean HAM-D 17 Item in patients was 24. Both morning and evening levels of DHEA were different between groups, with DHEA significantly lower in depressed compared to control groups. DHEA was also lower in the evening in depressed compared to patients in remission. Salivary cortisol was higher in depressed compared to other groups. Values for the remitted group were intermediate. DHEA levels at 0800 hrs correlated negatively with severity of depression and were not related to drug treatment or smoking, but as expected decreased with age (Michael et al., 2000).

In summary although DHEA/DHEA-S has been measured in various studies in depression similar problems arise as with cortisol. The sample is often poorly defined and various types of depression are included. Method of collection, time and co-morbidity are other problems that often exist that make interpretation of results difficult.

DHEA/DHEA-S and Treatment Resistant Unipolar Depression

Apart from the studies described above DHEA has never been measured in clearly defined TRUD and none of the above studies have attempted to define TRD.

DHEA/DHEA-S and abuse in (TR)UD

Daily plasma DHEA/DHEA-S has been found to be higher in patients with PTSD related to childhood abuse. Salivary DHEA morning levels have been found to be higher in subjects with Post Traumatic Stress Disorder and Borderline Personality Disorder (Jogems-Kosterman et al., 2006). Kellner and colleagues also found that DHEA/DHEA-S plasma levels increased in subjects with PTSD and childhood abuse (Kellner et al., 2010).

DHEA/DHEA-S and Bipolar Depression

Studies on DHEA and Bipolar Depression are lacking. Herewith is summarised the evidence to date.

Marx and colleagues measured DHEA post-mortem in 15 brains of patients with Bipolar Disorder, 14 brains of non psychotically depressed subjects and controls. Information on the number of episodes, severity of depression and criteria used to define depression is not provided in the study. It is not clear what was the course or type of Bipolar Disorder. DHEA was studied post-mortem in the posterior cingulate and the parietal cortex, which are amongst the brain areas linked to schizophrenia pathophysiology (Haznedar et al., 2004; Danckert et al., 2004). Median DHEA levels were significantly elevated in the posterior cingulate in bipolar disorder compared to controls, whereas depressed subjects had slightly higher DHEA levels than controls (median DHEA 16.35 in bipolar patients, 7.64 in depression and 5.68 in controls). A similar finding was apparent when the steroids were measured at the parietal cortex (median DHEA 18.33 ng/g in bipolar patients, 2.83 ng/g in depressed patients and 3.67 ng/g in controls). Since neuroactive steroids are often regulated differently in males and females, a separate statistical analysis was conducted between males and females revealing much higher DHEA levels in males compared to females in both areas (19.81 ng/g vs 11.68 ng/g in the posterior cingulate and 29.55 ng/g vs 12.17 ng/g in the parietal cortex). It is however unclear whether patients in this study were chronically unwell. Neurosteroid levels did not differ in subjects based on the use of antipsychotics, although it is not clear whether this is related to their use or to clinical response and thus firm conclusions about illness status cannot be drawn. The use of antidepressants was not evaluated (Marx et al., 2006).

It is therefore possible that these higher DHEA levels are a result of enhanced biosynthesis or compensatory upregulation of DHEA although the exact mechanism is not known, they could potentially alter the inhibitory and excitatory neurotransmission pathway, which could lead to disease.

DHEA and lithium treatment

Following the notion that DHEA/DHEA-S have mood elevating properties the impact of lithium treatment on serum and brain DHEA/DHEA-S was measured on the hippocampus and the frontal cortex in rats following lithium administration. Rats were divided in two groups one of which was fed with food containing lithium for ten days. Following this experiment rats were sacrificed and brain and serum tissue were extracted. There was a decrease in brain DHEA/DHEA-S up to 72% in the rats that consumed Lithium both in the frontal cortex and the hippocampus which differed between the two groups. Although lithium treatment did not change serum DHEA-S levels, it decreased DHEA levels. The lowering of DHEA both in the frontal cortex and hippocampus in lithium treated rats could be related to the mechanism of action of lithium (inhibition of PAP phosphatase and consequently elevated PAP levels resulting in inhibition of sulphation and reduction in brain DHEA/DHEA-S levels (Maayan et al., 2004).

DHEA/DHEA-S and abuse in (TR)BD

This has never been evaluated.

Cortisol/DHEA ratio

Unipolar Depression and Cortisol/DHEA ratio (plasma)

The ratio of Cortisol/DHEA has also been measured in patients suffering with comorbid depression and Borderline Personality Disorder. 12 unmedicated female patients who met DSM-IV criteria for Major Depressive Disorder and Borderline Personality Disorder and had a mean BDI of 35 were compared to 12 healthy women. Plasma (serum) of each participant was obtained during the follicular phase of the menstrual cycle between 1500 and 1900 hrs at 10-min intervals in order to obtain short cortisol profiles. Patients exhibited elevated serum cortisol concentrations and an increased Cortisol/DHEA ratio compared to controls (Kahl et al., 2006).

Unipolar Depression and Cortisol/DHEA ratio (saliva)

Michael and colleagues measured salivary cortisol and DHEA over a 4 day period in the morning and evening in 3 groups of patients suffering with Major Depressive Disorder, 35 partially remitted subjects and 41 controls. Although the ratio did not differ between the latter two groups, it was elevated in the depressive group both in the morning and in the evening (Michael et al., 2000).

The molar salivary ratio of Cortisol/DHEA was also found to be elevated in 39 medication-free depressed patients compared to 41 controls at 0800 and 2000 hrs (using salivettes). The majority of the depressed patients included in this study were experiencing their first depressive episode. The ratio of Cortisol/DHEA was found to be greater in depressed patients compared to controls. Furthermore the Cortisol/DHEA ratio at 0800 hrs positively correlated with the length of the current depressive episode (Young et al., 2002).

Unipolar Depression and Cortisol/DHEA ratio in children and adolescents (saliva)

The ratio of Cortisol/DHEA and its significance in adolescent depression as well as possible prediction of future depressive episodes has been evaluated in a series of studies by Goodyer and colleagues. To assess whether the ratio of Cortisol/DHEA predicted depression at onset and at 12 weeks of follow up this was measured in adolescents on presentation of their first depressive episode and at reassessment after 12 months. Saliva was collected at 5 time points, at 0800, 1200, 1600, 2000 and 2400 hrs. Higher Cortisol/DHEA ratios at 2000 or 2400 hrs predicted persistent major depression whereas basal levels of either hormone alone or the Cortisol/DHEA ratios at the other three time points did not. Looking at the Cortisol/DHEA ratio from a slightly different angle the authors reported that values greater than the 60th percentile at both evening points predicted the occurrence of subsequent disappointing life events (Goodyer et al., 1998).

To assess whether psychoendocrine factors predicted depression at 36 weeks, 181 adolescents at high risk for psychopathology were assessed. Cortisol, DHEA and their ratio were measured at entry and after 12 months over 4 days via the drooling method. Subjects meeting criteria for high risk at entry were reassessed at 1 year. High risk was defined by the presence of greater than 2 undesirable life events over the last 12 months (danger to self, others, disappointments, loss), current or past marital disharmony/breakdown, high emotionality, 2 or more lifetime exit events or that have personal significance to the adolescent and history of parental psychiatric disorder (Goodyer et al., 2000a).

Of the 172 reassessed subjects 30 met DSM-III-R criteria for depressive disorder.

Higher morning DHEA at entry was associated with onset of subsequent depression (Goodyer et al., 2000b). Depression was also predicted by hypersecretion as measured by peak positives for both morning and evening cortisol and DHEA. Peak was defined as whether or not a subject had a 'peak' that lay at or above the 80th percentile of the mean value for the group for each gender (and age for DHEA). This type of measure has been used to estimate variability or 'reactivity' in cortisol in children (Gunnar et al., 1996). Depression was indeed predicted by 1 or more peak values of cortisol at 0800 and DHEA at 2000 hrs (Goodyer et al., 2000).

To evaluate whether depression is predicted by endocrine values in the longer term Goodyer and colleagues measured salivary cortisol and DHEA at 0800 and 2000 hrs in subjects with a first episode of depression who were then reassessed after a duration of 36 and then 72 weeks. 360 8-16 year old adolescents were screened with a self report questionnaire for depressed mood and feelings (cut off score above 25). Of the 120 people that were above the cut off score of the questionnaire and therefore eligible for the study, 104 agreed to participate but only 78 of these met DSM-III-R criteria for depressive disorder. Of these 87% were reassessed at 36 weeks and 53 agreed to be reassessed at 72 weeks. Recovery from an episode was defined as having less than 2 clinically significant depressive symptoms for at least 8 weeks. Salivary cortisol and DHEA were obtained at 2 time points over 2 consecutive days. Of the original sample only 20 met DSM-III-R criteria at 72 weeks.

Hormones were collected via the drooling method which is shown not to interfere with DHEA levels. Depressed subjects exhibited higher evening cortisol compared to those

subjects that had recovered. No differences were found in the morning cortisol between groups. DHEA was also found to be lower at 72 weeks compared to entry and 36 weeks between the two groups. No changes were noted in the Cortisol/DHEA ratio between groups at different measurement points (Goodyer et al., 2001).

Goodyer and colleagues also investigated whether patterns of cortisol and DHEA that precede the onset of an episode of major depression influence time to recovery in a community ascertained sample of adolescents meeting DSM-IV criteria for Major Depressive Disorder. 60 adolescents aged 12 to 16 at high risk for psychiatric disorders were followed for 24 months. At 12 months, 30 had experienced an episode of major depression and 30 had not. Compared to the never depressed (N = 30) and remitted adolescents (N = 19), persistently depressed cases (N = 11) had a raised 0800 hrs salivary morning cortisol/DHEA ratio at entry. The raised Cortisol/DHEA ratio at entry was the only indicator of persistence of symptoms (Goodyer et al., 2003).

Finally the ratio of DHEA-S/Cortisol was evaluated in 12 depressed patients and 11 matched controls. Depressed patients with a diagnosis of major depressive disorder based on DSM-III-R were compared to 11 matched controls. Each patient was evaluated by a clinical interview including application of the HAM-D 17 Item (Hamilton, 1960). Patients received pharmacotherapy with clomipramine at a mean dose of 70 ± 50 mg/day for 1 month. Before treatment patients had significantly higher values of plasma DHEA-S and cortisol than those of normal controls. After the treatment plasma DHEA-S decreased significantly. Treatment with clomipramine did not change the ratio of plasma cortisol/DHEAS (Takebayashi et al., 1998).

Paslakis and colleagues reported no change in the Cortisol/DHEA-S ratio following a 4 week treatment period with venlafaxine and mirtazapine. In this study cortisol was measured at 0830 hrs in saliva with the use of salivettes, whereas DHEA-S was measured in plasma at 0800 hrs. 70 patients and 33 matched controls were included in the study. Diagnosis of Major Depression was according to DSM-IV criteria and patients had a minimum HAM-D 21 Item score of 18. Bipolar Disorder of Type I, but not other types of Bipolar Disorder, constituted an exclusion criterion for the study. The ratio of Cortisol/DHEA-S did not significantly differ between the two patient groups but was higher compared to controls. Post treatment, remitters treated with venlafaxine, but not those treated with mirtazapine had a higher Cortisol/DHEA-S ratio compared to controls. Among non remitters there was no significant change in the ratio.

Schule and colleagues found the ratio of Cortisol/DHEA to be unaltered following a 5-week treatment period with mirtazapine. This study has been previously described and in brief the ratio of Cortisol/DHEA was found to be unaltered following 5 weeks of mirtazapine treatment (Schule et al., 2009).

Therefore from the studies above it seems that the ratio of Cortisol/DHEA is a more consistent finding, as most of the studies report an elevated ratio in depression. Thus, if one were to accept that DHEA has an antiglucocorticoid activity as suggested, then had DHEA also increased alongside cortisol there may not have been an excess of glucocorticoid activity; however, the elevation of the Cortisol/DHEA ratio is likely to represent an excessive “net” glucocorticoid activity. This could make the ratio of Cortisol/DHEA a much more robust biological marker than either cortisol or DHEA individually.

The concept of using a ratio in medicine is not novel. There are several fields within medicine where a ratio of different variables is used in order to define the condition, such as polycystic ovarian syndrome, dyslipidemias and cardiovascular disease. Nevertheless, ratios do not replace the importance of understanding the components of that ratio; different factors of importance may affect differentially cortisol, DHEA and their ratio. Although this is the case what possibly makes the importance of measuring a ratio even stronger is that studies in affective disorders that have measured DHEA in isolation have been inconclusive.

Furthermore some studies have evaluated the ratio in predicting further depressive episodes, therefore the ratio may play a role in the field of prevention which is clearly of paramount importance in medicine.

Despite the above the same gaps as mentioned previously remain related to the definition of depression or that the ratio has never been evaluated in TRD.

Bipolar Depression and Cortisol/DHEA ratio (plasma)

The ratio of Cortisol/DHEA has been measured in plasma in 20 patients with Bipolar Disorder compared to controls. Samples were collected from 1300 to 1600 hrs in 20 patients with Bipolar Disorder and 20 controls recruited from secondary and tertiary care. As opposed to most studies measuring neuroendocrine steroids in affective disorders this sample included predominantly males (18 males and 2 females). Mostly although symptomatic subjects were stable on medication and were taking at least one mood stabiliser. Bipolar Disorder was confirmed using the Structured Clinical Interview for DSM-IV. 17 of the subjects included had a HAM-D 17 Item above 18 and 3 had a

HAM-D 17 Item score of 4. Therefore this study did not differentiate between remitted and acutely unwell subjects.

Cortisol was found to be higher in bipolar patients compared to controls. DHEA did not differ between patients and controls. The molar ratio of Cortisol/DHEA did not show a significant main effect of diagnosis (Gallagher et al., 2007).

Bipolar Depression and Cortisol/DHEA ratio (saliva)

This has never been evaluated.

Treatment Resistant Bipolar Depression and Cortisol/DHEA ratio

The ratio of Cortisol/DHEA in TRBD has never been evaluated.

AIMS AND OBJECTIVES

The aim of the project is to measure the HPA axis activity in two conditions, TRUD and TRBD. This is done in two different ways: first, by using a dynamic measure of the capacity of the HPA axis to respond to challenge, the CAR; and second by measuring the diurnal variation of cortisol and DHEA and calculating the Cortisol/DHEA ratio in the morning, noon and evening. For each of the above measures, comparisons are made: between patients during the acute phase of their illness and in remission; and between patients and controls. Although the focus of the project is to use saliva to assess the HPA axis, as a novel, easy, non invasive tool to measure biological activity, plasma samples have also been used in the development, design and execution of the project.

Given its complexity and the fact that different subject groups are included this project is divided in the following series of three studies:

CAR and Cortisol/DHEA in TRUD measured in plasma, abbreviated as *Study I*, *TRUD/plasma*.

CAR and Cortisol/DHEA in TRUD measured in saliva, abbreviated as *Study II*, *TRUD/saliva*.

CAR and Cortisol/DHEA in TRBD measured in saliva, abbreviated as *Study III*, *TRBD/saliva*.

Study I, Treatment Resistant Unipolar Depression/plasma

The aim of this study is to measure plasma cortisol, DHEA and the ratio of Cortisol/DHEA in patients with TRD.

Comparisons are made:

1. Between patients during the acute phase of their illness and healthy controls.
2. Between patients during the acute phase of their illness and in remission.
3. Between patients in remission and controls.

This study formed the pilot work for the series of studies in the PhD. Thus a smaller number of patients and controls was used, and the study used only single plasma measurements. Nevertheless, this was the first study that attempted to measure cortisol, DHEA and their ratio in TRUD. Since it was felt that the CAR would give more information with regards to cortisol activity as opposed to single cortisol measurements,

and that multiple measures using salivary cortisol as a novel non invasive way of measuring cortisol would be preferable, the studies below were consequently designed.

Study II, Treatment Resistant Unipolar Depression/saliva

The aim of this study is to measure a. the salivary CAR b. diurnal cortisol and DHEA, c. the ratio of Cortisol/DHEA in patients with TRUD. The following comparisons are undertaken:

1. Between patients during the acute phase of their illness and healthy controls.
2. Between patients during the acute phase of their illness and in remission.
3. Between patients in remission and controls.

Study III, Treatment Resistant Bipolar Depression/saliva

To measure a. the salivary CAR b. diurnal cortisol and DHEA, c. the ratio of Cortisol/DHEA in patients with TRBD the following comparisons are undertaken:

1. Between patients during the acute phase of their illness and healthy controls.
2. Between patients during the acute phase of their illness and in remission.
3. Between patients in remission and controls.

Hypotheses under investigation

The hypotheses under investigation derive from the findings of the pilot study (*Study I, TRUD/plasma*) where plasma cortisol, DHEA and their molar ratio were compared in 29 treatment resistant depressed patients and 40 controls. According to this study there

was an increased Cortisol/DHEA ratio, lowered DHEA and hypercortisolemia in patients compared controls. Based on this the following are hypothesised:

1. Comparison of salivary cortisol, DHEA and the Cortisol/DHEA ratio between patients with TRUD, in remission and controls, in the morning, afternoon and evening. The salivary Cortisol/DHEA ratio will be increased between patients in the acute phase of their illness compared to those in remission and in controls.
2. Comparison of salivary cortisol, DHEA and the Cortisol/DHEA ratio in patients with TRBD, in remission and in controls, in the morning, afternoon and evening. The starting hypothesis is that there will also be an enhanced Cortisol/DHEA ratio and hypercortisolemia in the acute phase compared to remission and controls. However there is a possibility that Bipolar Depression presents with a different neuroendocrine profile to Unipolar Depression, and if this is the case it would be hypothesised that there will be no change in cortisol secretion or even reduced cortisol levels may be found (as also found in a pilot study by Rasgon et al., 2007).
3. CAR in TRUD. There will be an enhanced and prolonged CAR in currently depressed patients relative to those in remission and to healthy controls. In remitted patients, the magnitude of the CAR will be decreased compared to non remitters.
4. CAR in TRBD. If we hypothesise that in Bipolar Depression there are the same biological abnormalities as in Unipolar Depression then it is likely that there will

be an enhanced and prolonged CAR in currently depressed patients relative to those in remission and to healthy controls (in remitted patients, the magnitude of the CAR will be decreased compared to non remitters). However there has been no study to date that has attempted to measure the above abnormalities in clearly defined TRBD, thus there is a possibility that TRBD presents will have a different biologic profile compared to TRUD and show a normal or reduced CAR.

5. A final hypothesis is that the Cortisol/DHEA ratio will be predictive of treatment response, and that early relapse will be predicted by a failure of the Cortisol/DHEA ratio to normalise.

The research questions and relevant studies that comprise this thesis and the way they were gradually designed are the following:

The preliminary study that was designed was based on measuring morning plasma cortisol, DHEA and their ratio in inpatients with TRUD. Measurements were at baseline and following a period of inpatient treatment after which cortisol, DHEA and their ratio were repeated in treatment responders and non-responders.

Following this pilot study a larger study was designed to measure the salivary Cortisol Awakening Response (abbreviated as CAR) as well as cortisol, DHEA and their ratio in the morning, noon and evening between acutely unwell inpatients and outpatients suffering with TRUD and TRBD, subjects in remission and controls.

The thesis consists of 4 chapters. In this chapter the body of literature work has been described in detail as well as further specifics related to the HPA axis, the

Neuroendocrinology of cortisol and DHEA, the Neurobiology of TRUD and TRBD and the advantages of salivary use compared to other methods of hormone collection. The methodology will be outlined in Chapter 2. A description of the results will follow in Chapter 3 and in Chapter 4 these will be discussed together with recommendations for future research work.

CHAPTER 2: METHODOLOGY

RECRUITMENT

Patients

Recruitment of subjects was predominantly through/via the National Affective Disorders Services, at the South London and Maudsley NHS Foundation Trust; local mental health services mainly the Community Mental Health Team at Southwark (an inner city area in London) were also used to supplement recruitment. Patients included in the studies were aged between 18 and 75.

The National Affective Disorders Unit

The National Affective Disorders Service consists of two parts: the Outpatient Service and the Inpatient Affective Disorders Unit. Referrals are from secondary care nationwide. Almost all of the Inpatient admissions follow assessment in the Outpatient Service. Admissions are planned and organised weeks or sometimes months in advance. Inpatient admissions are usually recommended for a further assessment period. They are recommended in cases which present as clinical dilemmas to the referring agents and/or where changes in therapy should preferably be provided in an inpatient setting to avoid complications/ensure monitoring. Most patients admitted to the ward are chronically unwell.

The ward provides pharmacological expertise, a diagnostic opinion and an opportunity to participate should they wish to in psychological, couple therapy and a plethora of occupational activities to facilitate behavioural activation.

Diagnosis/inclusion/exclusion criteria

Definition and Staging of Treatment Resistance

Patients recruited have not been responsive to at least two or more treatments provided locally at the minimum therapeutic dose and duration and are diagnosed as TRD as defined by the The Massachusetts General Hospital staging method (MGH-S) and the Antidepressant Treatment History Form (Sackeim, 2001). Other studies have defined occurrence of treatment-resistance as a failure to respond to at least one antidepressant medication (Thase and Rush, 1997), or as absence of remission (Greden et al., 2001). The current study defined TRD as a failure to respond to at least 2 previous adequate trials of standard classes of antidepressants (at least 6 week adequately dosed trials, with or without augmentation (Sachs, 1996).

Regarding TRBD, subjects with Bipolar Disorder were only recruited during the depressed phase of their illness. They had failed to respond to at least 2 previous adequate trials of standard classes of antidepressants (at least 6 week adequately dosed trials, with or without augmentation (Sachs, 1996). Thus, they were also classified as Treatment Resistant based on the criteria above for the depressive phase of their illness.

In addition, Thase and Rush criteria (Thase and Rush, 1995) were used to stage the degree of resistance, for the plasma study.

Although almost all patients with TRUD (90%) and TRBD (95%) had recurrent depression they were also clearly treatment resistant.

Inclusion Criteria

Study I, Treatment Resistant Unipolar Depression/plasma

Patients were included in the study if they were an Inpatient/Outpatient of the Affective Disorders Services, aged between 18 and 75 and diagnosed as having unipolar major depressive disorder according to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, DSM-IV (American Psychiatric Association, 1994). In addition they were diagnosed as having TRD, namely having been resistant to treatment with at least two different classes of antidepressants at the minimum therapeutic dose (as defined by Sackeim, 2001). Being on psychotropic medication was not an exclusion criterion.

Study II, Treatment Resistant Unipolar Depression/saliva

Patients were included in the study if they were an Inpatient/Outpatient of the Affective Disorders Services, aged between 18 and 75 and diagnosed as having unipolar major depressive disorder according to the International Classification of Diseases, 10th Edition, ICD-10. In addition they were diagnosed as having TRD, namely having been resistant to treatment with at least two different classes of antidepressants at the minimum therapeutic dose (as defined by Sackeim, 2001). Being on psychiatric medication was not an exclusion criterion.

Study III Treatment Resistant Bipolar Depression/saliva

Patients were included in the study if they were an Inpatient/Outpatient of the Affective Disorders Services, aged between 18 and 75 and diagnosed as having unipolar major depressive disorder according to the International Classification of Diseases, 10th Edition, ICD-10. Patients had a previous diagnosis of Bipolar Affective Disorder as defined by ICD-10. In addition they were diagnosed as having TRBD, namely having been resistant to treatment with at least two different classes of antidepressants at the minimum therapeutic dose (Sachs, 1996). Psychiatric medication was not an exclusion criterion.

Controls

Controls aged between 18 to 75 were included in the study from our local database.

Controls and their first degree relatives were never diagnosed to be suffering from any form of psychiatric illness. Controls were recruited from our database of volunteers which included hospital and university staff and students and also members of the local community. Psychiatric illness was assessed by direct questioning of the controls and by the administration of screening questionnaires (BDI). Controls were also asked whether they had a first degree relative suffering from a mental health illness such as depression, anxiety, alcoholism and so on. Controls were matched with patients according to their age \pm 5 years, gender and BMI, the latter calculated as weight divided by height² (BMI \pm 5 kg/m²).

Exclusion Criteria

Study I, Treatment Resistant Unipolar Depression/plasma

Study II, Treatment Resistant Unipolar Depression/saliva

1. Any major organic disorder that could explain the psychiatric diagnosis.
2. Any systemic physical illness or neurological condition that could affect the hormonal concentrations measured.
3. Regular corticosteroid use.
4. Heavy smoking (more than 40 cigarettes/day).
5. Alcohol dependence or drug use/dependence. Abstinence from alcohol for the previous 7 days and throughout the protocol. No alcohol dependence syndrome in the last 6 months (Posener et al., 2000).
6. Pregnancy/ lactation.

Controls

1. Any systematic current physical illness or neurological condition that could affect the hormonal concentrations measured.
2. Any past or present psychiatric diagnosis as defined by DSM- IV.
3. Any psychiatric condition in first degree relatives.
4. Any medication that could affect hormonal levels and particularly corticosteroids.
5. Pregnancy and use of illicit drugs.
6. A HAM-D 21 Item above 10.
7. Heavy smoking (more than 40 cigarettes/day)

Medical screening for subjects

Patients were screened to exclude a detectable organic cause for their illness by detailed history, physical examination, blood testing (including C-reactive protein, urea and electrolytes, full blood count, thyroid function tests, autoantibodies, liver function tests and B12 and folate levels) and brain MRI. None of the included patients were using alcohol in excess of recommended limits (21 units/week for women and 28 for men).

Place of study

The place where the study was undertaken was either the Inpatient Affective Disorders Unit or the subjects' home. Those who decided to take part in the study at home were posted the relevant instructions, questionnaires and saliva kit with instructions (see appendix 2) on collecting the samples and an envelope in order to post the samples to the laboratory. Further support and instructions were given to them telephonically. All patients were assessed during the depressive phase of their illness or during remission from the depressive phase. Patients did not start inpatient treatment free of medication. In some cases treatment was discontinued following an initial inpatient assessment if deemed to not be appropriate or not working. Soon after, a new treatment was initiated. Almost all patients included in the study were on medication at the time of testing.

Patients were re-assessed clinically after a period of individually-optimised inpatient treatment including psychopharmacotherapy based on the Maudsley Prescribing Guidelines 8th and 9th edition (Taylor et al., 2005, 2008).

Ethical approval

All procedures were approved by the hospital ethics committee. Patients and

controls gave written informed consent prior to taking part to the testing.

CLINICAL INSTRUMENTS UTILISED

Here the clinical instruments in the studies utilised are described. As mentioned in Chapter 1 the studies that this thesis describes are those below:

1. Cortisol/DHEA in Treatment Resistant Unipolar Depression measured in plasma, abbreviated as: Study I, Treatment Resistant Unipolar Depression/plasma
2. CAR and Cortisol/DHEA in Treatment Resistant Unipolar Depression measured in saliva abbreviated as: Study II, Treatment Resistant Unipolar Depression/saliva
3. CAR and Cortisol/DHEA in Treatment Resistant Bipolar Depression measured in saliva abbreviated as: Study III Treatment Resistant Bipolar Depression/saliva

Diagnostic Assessment

Structured Clinical Interview for DSM-IV Axis I disorders – SCID-I (First et al., 1997)

Study I, Treatment Resistant Unipolar Depression/plasma

The diagnostic criteria from DSM-IV (American Psychiatric Association, 1995) were applied in order to clearly define the current depressive episode. For diagnostic purposes, interviews were by trained clinicians.

Subjects eligible for inclusion in the unipolar depression group met DSM-IV criteria for Major Depressive Disorder or Major Depressive Episode as the primary psychiatric disorder.

The Structured Clinical Interview for DSM- IV (SCID-I) a semi-structured interview for making the major Axis I DSM- IV diagnoses, was administered to all patients. This includes an introductory overview followed by nine modules, seven of which represent the major axis I diagnostic classes. Because of its modular construction, it can be adapted for use in studies in which particular diagnoses are not of interest. Using a decision tree approach and the SCID guidelines, the Interview was focused on the Mood Disorders Section. SCID- I was assessed for current episode (past month) and for lifetime occurrence. Although the SCID provides structure to cover criteria for each disorder, it allows for flexibility, in that the interviewer can (1) probe and restate questions (2) challenge the respondent (3) ask for further classification if necessary to make a determination as to whether a particular symptom of a disorder is present.

International Classification of Diseases - ICD-10 Criteria

Study II Treatment Resistant Unipolar Depression/saliva

Study III Treatment Resistant Bipolar Depression/saliva

The International Classification of Diseases is published by the World Health Organization and used worldwide for morbidity and mortality statistics, reimbursement systems and automated decision support in medicine. This system is designed to promote international comparability in the collection, processing, classification, and presentation of these statistics. The ICD is a core classification of the WHO Family of International Classifications (WHO-FIC).

Subjects eligible for inclusion in the unipolar depression group met ICD-10 criteria for Major Depressive Disorder or Major Depressive Episode as the primary psychiatric disorder. Those included in the TRBD met criteria for a diagnosis of Bipolar Affective Disorder currently suffering with a depressive episode that was resistant to treatment.

The ICD is revised periodically and is currently in its tenth edition. The ICD-10, as it is therefore known, was developed in 1992 to track mortality statistics. Annual minor updates and three-yearly major updates are published by the WHO. The ICD is part of a "family" of guides that can be used to complement each other, including also the International Classification of Functioning, Disability and Health, which focuses on the domains of functioning (disability) associated with health conditions, from both medical and social perspectives.

Study I was part of a larger study and used the SCID to generate DSM-IV diagnoses. For the latter studies, we preferred to use the ICD-10 as generated by clinician ICD-10 interview, supplemented in about half of the cases with the Mini International Neuropsychiatric Interview (MINI), both generating ICD-10 diagnoses, as this is the standard categorisation used within the UK and in our institution. The ICD-10 and MINI interview were carried out by a trained psychiatrist from the SLAM NHS Trust (KM).

Structured Clinical Interview for Personality Disorders –SCID-II (First et al., 1995)

Study I, Treatment Resistant Unipolar Depression/plasma

The SCID-II questionnaire was used to assess comorbidity of personality disorders. If the personality disorder is felt by the clinician to be the primary diagnosis then the

patient is not included in the study. (The SCID-II questionnaire was also used in studies II and III, as an additional aid of personality disorders, taking however into account the risk of overdiagnosis). The SCID-II questionnaire examines the presence of more than one personality disorder it is therefore possible for the same patient to suffer from more than one comorbid personality disorder. The different personality disorders that are measured by the SCID-II questionnaire are the following:

Dependent personality disorder

Borderline personality disorder

Narcissistic personality disorder

Obsessive compulsive personality disorder

Schizoid personality disorder

Avoidant personality disorder

Passive aggressive personality disorder

Schizotypal personality disorder

It therefore includes the ten personality disorders specified by DSM-IV and two appendix categories. Due to poor discriminant validity of the constructs and possibly assessments there is a big overlap between diagnoses and often patients receive more than one diagnoses. An interview follows the questionnaires.

Clinical Severity of Depression

Hamilton Rating Scale for Depression (HRSD) or Hamilton Depression Rating Scale (HDR) or HAM-D) 21 Item Version (Hamilton, 1960)

Study I, Treatment Resistant Unipolar Depression/plasma

Study II, Treatment Resistant Unipolar Depression/saliva

Study III Treatment Resistant Bipolar Depression/saliva

The Hamilton scale (Hamilton 1960) was applied to all patients by a trained psychiatrist to assess severity of depression by a trained psychiatrist. The 21 Item scale was used. Subjects included had a cut off score ≥ 17 . Psychiatrists that administered the HAM-D had been trained as mentioned above and also used the structured format of the scale (Williams et al., 1988) to further aid the measuring according to Hamilton recommendations published in 1967.

Subjects were classified as responders or non responders based on a 50% reduction of the HAM-D 21 Item Rating Scale at discharge point (Keller, 2003). Remission was classified as a HAM-D of 10 points or less at the point of discharge or when subjects were re-tested.

The primary outcome measure to assess symptomatic improvement was the 21 Item Hamilton Rating Scale for Depression (HAM-D) (Hamilton, 1960) a semi-structured, clinician-administered scale regarded as the “gold-standard” in the field. We used a structured interview guide for this scale to aid reliability (SIGH-D) (Williams, 1988). We defined *a priori* remission as a discharge HAM-D score of 10 points or less (equivalence charts available in multiple languages at (<http://www.ids-qids.org/index2.html#table3>)), a score which has been previously used in this population (Wooderson et al., 2011) and in other studies (Kummer et al., 2010). There is no universally accepted definition of remission, and it is the case that some other studies have used a cut off on the 17 Item HAM-D of 7 or 8 points or less (equivalent to 8 or 9 points on the 21 Item HAM-D). Our choice of a less stringent cut-off also reflected what we felt was a more realistically achievable treatment response in a group

of largely chronically ill patients. Only one patient with TRBD and 2 patients with TRUD had a HAM-D 21 Item of 10. Using the 21 Item scale gave us more information on atypical symptoms such as reversed diurnal variation.

Stage of Treatment Resistance

Thase and Rush staging criteria (Thase and Rush, 1997)

Study I, Treatment Resistant Unipolar Depression/plasma

According to the above criteria, severity of treatment resistance in depression is divided in 5 stages which are the following:

Stage 1: No response despite an adequate therapeutic trial

Stage 2: No response to two therapeutic trials of medication of two different medications

Stage 3: failure to respond to two antidepressant trials and one augmentation strategy

Stage 4: failure to respond to two different antidepressant trials and two augmentation strategies

Stage 5: failure to respond to stage 4 plus ECT

Antidepressant Treatment History Form (Sackheim et al., 1990)

Study I, Treatment Resistant Unipolar Depression/plasma

Prior to hospitalization a history of treatment of depression was utilized and the antidepressant treatment history form was also used to confirm which patients have been resistant to treatment with at least two different classes of antidepressants used at a minimum or greater therapeutic dose and duration.

Life Events Questionnaire (Brugha et al., 1985)

Study II Treatment Resistant Unipolar Depression/saliva

Study III Treatment Resistant Bipolar Depression/saliva

Life events are usually short-lived but may have more enduring consequences. They can be distinguished from 'chronic difficulties', such as poverty or persistently discordant relationships. However life events can be both an indicator of chronic difficulties, or a precipitant of them. Negative life events have the capacity to affect any family member not just those directly involved.

An important issue is whether an event is felt to continue to exert a negative affect. This aspect has not always been included in questionnaires.

The Life Events questionnaire utilized has been developed from one devised by Brugha et al (1985), with 9 additional items. The scale aims to look at recent life events, those occurring in the last 12 months and whether the respondent thinks they have a continuing influence.

Impact of Events Scale (Horowitz et al., 1979)

Study II Treatment Resistant Unipolar Depression/saliva

Study III Treatment Resistant Bipolar Depression/saliva

This is a 15-item self report scale which is used to assess the impact that a trauma has had on a person and can be used in the work-up of people with Post-Traumatic Stress Disorder (as well as related conditions) who undergo treatment (Horowitz et al, 1979). Therefore it was used to assess PTSD symptoms. This instrument, evaluates the distress

that is caused by traumatic events. The test is centered around two subscales – Intrusion, Avoidance, The IES-R is the revised edition of the original IES.

The format for the test is a 15-item self-report in which respondents identify a stressful event and then respond to question measuring distress with a 4-point scale. Presented by Solomon & Mikulincer (1988), the test-retest reliability for IES was estimated at 0.56 - 0.74, based on a two different samples.

Perceived stress scale 10 Item (Cohen et al. 1983)

Study II Treatment Resistant Unipolar Depression/saliva

Study III Treatment Resistant Bipolar Depression/saliva

The 10-item of this self-report instrument with a five-point scale was used:

(0 = never, 1 = almost never, 2 = sometimes, 3 = fairly often, 4 = very often).

It measures the degree to which situations in one's life over the past month are appraised as stressful. Items were designed to detect how unpredictable, uncontrollable, and overloaded respondents find their lives. It poses general queries about relatively current levels of stress experienced. All items begin with the same phrase: In the past month, how often have you felt...?

The PSS was designed for use with community samples with at least a junior high school education. The items are easy to understand and the response alternatives are simple to grasp. Moreover, as noted above, the questions are quite general in nature and hence relatively free of content specific to any sub population group

Functional capacity and adaptation

SF-12 (Ware JE, Kosinski M, and Keller SD. A 12-Item Short-Form Health Survey)

Study II Treatment Resistant Unipolar Depression/saliva

Study III Treatment Resistant Bipolar Depression/saliva

The SF-12 was designed to measure general health status from the patient's point of view. The SF-12 includes 8 concepts commonly represented in health surveys: physical functioning, role functioning physical, bodily pain, general health, vitality, social functioning, role functioning emotional, and mental health. Results are expressed in terms of two meta-scores: the Physical Component Summary (PCS) and the Mental Component Summary (MCS).

The SF-12 is scored so that a high score indicates better functioning.

Sleep

Pittsburgh Sleep Quality Index-PSQI (Buysse et al., 1989)

Study I, Treatment Resistant Unipolar Depression/plasma

Study II, Treatment Resistant Unipolar Depression/saliva

Study III Treatment Resistant Bipolar Depression/saliva

The PSQI is a self-rating questionnaire resulting in a global score between 0 and 21, which consists of seven subscores. The questionnaire is easy to handle and can be completed within 5 min. The German version has been translated from English into German and then retranslated for comparison with the original version. In the German version, the estimate period was reduced from 4 to 2 weeks, because the German PSQI

served as an instrument to evaluate a short-term therapy for primary insomnia, for which the 4-week period seemed too long. The PSQI has a high sensitivity and specificity for insomnia patients in comparison to healthy controls, thus underscoring that it is a good measure for differentiating between good sleepers and patients suffering from sleep disturbances. It was developed in order to provide a reliable, standard measure of sleep quality, discriminate between good and poor sleepers. It is easy to administer and can be completed in a brief period of time. The questionnaire was developed by combining previous sleep reviews, clinical experience with patients who suffered with sleep disorders and survey type questionnaires. It comprises 19 self rated and 5 questions for the bed partner which are not used in the scoring of the questionnaire and are only used for clinical information. It thus results in a global score between 0 and 21, which consists of seven subscores, each weighed from 0-3. The highest the overall score the worst the sleep quality. The individual components assessed are duration of sleep, latency of sleep and quality of sleep, as well as sleep disturbances and use of medication.

The questionnaires that were used are included under Appendix 2.

ENDOCRINE PROTOCOL

Plasma specimen collection

Study I, Treatment Resistant Unipolar Depression/plasma

Patients refrained from eating or drinking from 10 pm onwards of the night prior to testing. At 0900 hrs the following morning, a blood sample was withdrawn following venepuncture. 10 ml of blood was collected in sodium heparin tubes and after centrifugation at 2500g the plasma was separated and frozen at -40°C before final analysis. In a sub-sample of 15 subjects the stability of cortisol/DHEA ratios across the day was assessed with 2 hourly samples from 0900-1700 hrs.

Saliva specimen collection

Study II, Treatment Resistant Unipolar Depression/saliva

Study III, Treatment Resistant Bipolar Depression/saliva

Subjects (patients and controls) were asked to provide 8 saliva samples in a day in plain polypropylene tube by using the drooling method. Plastic straws were available to aid with the collection. The reason for using the drooling method is that although for cortisol levels, all methods of saliva collection either using the salivettes or the drooling method correlate highly with plasma levels and with each other, for DHEA levels, only saliva samples collected using the drooling method correlate with plasma levels (Gallagher et al., 2006).

Time, date and duration of collection

Subjects were instructed to collect 6 specimens of saliva in the morning, the first sample immediately following awakening and thereafter at 15, 30, 45, 60 and 90 minutes on two consecutive days. In addition they were asked to collect another two specimens, one at noon and the other at 2200 hrs. If for any reason they were unable to produce a complete set of 8 specimens during any of the days they were asked to continue collecting specimens for a period of up to 4 days until they had collected a complete set of 2 days for the CAR. However for the morning, noon and evening ratio subjects were instructed to collect samples over 4 days.

Subjects were asked to make note of the collection day. Weekdays were defined as Monday through Thursday, and weekends were defined as Friday through Sunday. Friday was included in the weekend, since activities (e.g., going out with friends) in the evening of Friday are more consistent with weekend than weekday (Broderick et al., 2004). However, it is arguable that the distinction between work and non work days is not particularly relevant to this population as the large majority of patients were not in active employment.

Instructions for collection

Subjects were given a full set of tubes for 4 days. Details on time and duration of collection were given to subjects in writing in a protocol, which explained as simply possible /in simple terms the scientific reasons for undertaking the study. Subjects were instructed not to brush their teeth or have anything to eat or drink for at least an hour before the collection of the samples. Although the actual time of the morning collections of the samples was not controlled for, subjects were clearly instructed once

they were awake to stay relaxed by their beds, rinse their mouth, and start collecting saliva without undue delay. They were also instructed to avoid extremes in the time collections (before 0600 hrs and after 1000 hrs). Subjects were instructed to remain still for the duration of the collection and to try to avoid any form of stress for the whole duration of the testing period including undertaking any activities such as strenuous leisure activities, late nights and use of alcohol. In cases where stress was unavoidable subjects were requested to record such strenuous and stressful events in the relevant time sheets.

In order to control for the time that the patients gave the samples and to ensure that other confounders for the Cortisol and DHEA Awakening Response were not missed patients were administered 4 forms, one for each of the days they collected the salivary samples. Each of the form clearly stated the time intervals that the samples should be collected. Next to this, subjects were instructed to fill in the time samples were given and to briefly comment on their activities and mood state during the collection. Subjects were instructed to add in the forms whether they experienced any form of physical pain or any other information that they could think would be of relevance and that could be interfering with the study and the protocol.

Inside the packs parafilm was included which subjects were instructed to use in case of a dry mouth. Subjects on the ward were instructed to hand in the daily samples to a member of staff who then put them in the freezer. Subjects from home were told to put the specimens in their freezer once they finished with the collection.

Subjects that did the study from home were provided with a prepaid envelope in order to post the samples back to the Affective Disorders Unit.

The instructions and collection forms are included in the Appendix 2.

HORMONE MEASUREMENT

This is described in detail in Appendix 1. Briefly:

Plasma and saliva measurements of hormonal concentrations

Cortisol and DHEA concentrations in plasma and saliva were determined by competitive immunoassay (LIA) techniques.

Plasma DHEA was assayed in duplicate using a Luminescence immunoassay (IBL, Hamburg, Germany). If the difference (coefficient of variance – CV) between the duplicate measurements was higher than 10%, the analysis was repeated. The minimal detectable concentration was 0.4 nmol/l. DHEA results were converted in nmol/l by multiplying the initial value by 0.00347.

Plasma cortisol concentrations were measured using the DSL-2100 “Active Cortisol” Radioimmunoassay (RIA) Kit (Diagnostic Systems Laboratories). Intrassay precision was 6% at 74nmol/l and 5% at 360 nmol/l. Minimal detectable concentration was 14 nmol/l.

The principles of the immunoassays used are described in the appendix 1.

STATISTICAL ANALYSES

All parametric values are presented as means (\pm standard deviation, SD) and for non parametric values as median and inter-quartile range. All p-values reported are two-tailed. A value of $p < 0.05$ was considered as statistically significant. Data were analysed using the Statistical Package for Social Sciences, Version 15.0 (SPSS Inc.). Tests used for the individual studies were as follows.

Study I, Treatment Resistant Unipolar Depression/plasma

In this preliminary pilot study cortisol, DHEA and their ratio were compared between patients and controls as well as in patients pre and post treatment. Post treatment comparisons were undertaken between responders and non-responders.

Not all data were normally distributed. Non-normal data was transformed using a natural log transformation prior to analysis, though for clarity the original values are described in the results chapter. All statistical analyses for between group comparisons were undertaken using an independent t-test. For within group comparisons, to assess the effect of intervention, a paired t test was used. Correlational analysis was undertaken using Pearson or Spearman Correlation Coefficients depending on whether the data were normally distributed or not. For the analysis of hormone values across the day, we used a repeated measures analysis of variance ANOVA with a Huynh-Feldt correction.

Study II, Treatment Resistant Unipolar Depression/saliva

Study III, Treatment Resistant Bipolar Depression/saliva

Primary measures

Cortisol Awakening Response (CAR) - Area Under the Curve (AUC)

The first aim was to measure the total secretion of both cortisol and DHEA and the functional cortisol level (as measured by the Cortisol/DHEA ratio) following awakening.

The overall cortisol and DHEA response were measured by calculating the area under the saliva hormone level by time (0 to 90 mins) curve

- a. with respect to the ground (AUCg) and
- b. with respect to the increase (AUCi).

The former is thought to reflect the overall cortisol/hormone production and the latter to indicate the increase in hormone secretion. Both these formulae are derived from the trapezoidal method (Pruessner et al., 2003).

The two formulae used for the calculations were:

$$\text{AUCi} = 15 * (\text{mean1} + \text{mean2}) / 2 + 15 * (\text{mean2} + \text{mean3}) / 2 + 15 * (\text{mean3} + \text{mean4}) / 2 + 15 * (\text{mean4} + \text{mean5}) / 2 + 30 * (\text{mean5} + \text{mean6}) / 2$$

$$\text{AUCg} = (15 * (\text{mean2} - \text{mean1}) / 2) + (15 * ((\text{mean2} - \text{mean1}) + (\text{mean3} - \text{mean1})) / 2) + (15 * ((\text{mean3} - \text{mean1}) + (\text{mean4} - \text{mean1})) / 2) + (15 * ((\text{mean4} - \text{mean1}) + (\text{mean5} - \text{mean1})) / 2) + (30 * ((\text{mean5} - \text{mean1}) + (\text{mean6} - \text{mean1})) / 2),$$

where mean 1 is the value at 0 mins post awakening, mean 2 is the value at 15 mins post awakening, mean 3 is the value at 30 mins post awakening, mean 4 is the value at 45 mins post awakening, mean 5 is the value at 60 mins post awakening and mean 6 is the value at 90 mins post awakening.

In those cases where there were missing values in the above formulae, other than at 0 and 90 mins, the mean value of the two proximal time points was used. Where either 0 or 90 min values were missing, the AUC could not be calculated.

Cortisol Awakening Response - Mean levels

Mean levels of cortisol, DHEA and their ratio were also compared at each time point between groups using an independent t test if data were normally distributed and Mann-Whitney U test for data that were not normally distributed.

Daily secretion - Area Under the Curve (AUC)

The daily cortisol and DHEA total output, and that of functional cortisol (as measured by ratio of cortisol/DHEA) was calculated by measuring the area under the hormone concentration by time curve as follows:

$$AUC_g = (5 * (\text{mean}_0 + \text{mean}_{\text{noon}}) / 2) + (10 * (\text{mean}_{\text{noon}} + \text{mean}_{10\text{pm}}) / 2)$$

where mean 0 is the salivary value on awakening (0700 hrs), mean noon is the salivary value at noon and mean 10 pm is the value at 10 pm.

The average of 4 days for each subject was used in any group comparisons.

Other measures

To look at the relation between biological values and clinical measures a correlation analysis using Pearson's product–moment coefficients was applied, except for non normally distributed data where the Spearman test was applied.

For the correlations, a correction for multiple comparisons was applied using the rough false discovery rate – i.e. the α - value was adjusted by $(n+1)/2n$, which for 84 tests gave an adjusted significance level of $p < 0.025$. Only correlations below that value were deemed to be significant and only these are reported in Chapter 3.

CHAPTER 3: RESULTS

STUDY I, TREATMENT RESISTANT UNIPOLAR DEPRESSION/PLASMA

Design

This study served as a preliminary ‘pilot’ study for the rest of the studies that are included in the thesis. The endocrine protocol of this study is described under Chapter 2, Methodology.

Plasma cortisol, DHEA and the Cortisol/DHEA ratio were determined at 0900hrs in 28 patients with TRUD and 40 healthy controls. In 21/28 of the above patients the same measures were repeated following inpatient treatment and related to the outcome of such treatment. The stability of the Cortisol/DHEA was assessed with 2 hourly samples from 0900-1700hrs in a subgroup of 15/40 controls.

All patients had a primary diagnosis of Major Depressive Disorder and were recruited into the study from the Inpatient service of the National Affective Disorders Unit, at the South London and Maudsley NHS Trust. Patients were interviewed using the Structured Clinical Interview for DSM-IV (SCID) by a trained psychiatrist. Patients eligible for inclusion met DSM-IV criteria for Major Depressive Disorder (American Psychiatric Association, 1995) as the primary psychiatric disorder.

Results of Clinical Assessment

Demographics

All patients were of British origin. Results are given as Mean \pm SD unless specified. Age of onset of illness was 33 \pm 14 years. Regarding patients' illness histories, the duration of current illness episode was 38 \pm 29 months and the total illness duration was 18 \pm 13 years. The number of previous episodes was 5 \pm 4 and the age at onset of current episode was 45 \pm 11 years. The duration of admission was 23 \pm 14 weeks. 23/28 patients had previously had Electroconvulsive Treatment (ECT).

Details of patients' illness characteristics, as well as those of controls, are summarised in table 1. There were no significant differences between patients and controls in age ($t=1.88$, d.f.=66, $p=0.06$), Body Mass Index ($t=1.47$, d.f.=66, $p=0.15$) and gender ($\chi^2=1.52$, d.f.=1, $p=0.13$).

Season

Of the 28 patients on admission 6/28 were tested in spring, 9/28 in summer, 3/28 in autumn and 10/28 in winter. Of the 21/28 patients tested after treatment, 6/21 were tested in spring, 8/21 in summer, 3/21 in autumn and 4/21 in winter. No statistically significant differences were found between patients tested at different seasons.

Co-morbidity

The SCID interview was applied to all subjects to assess comorbidity. It was found that 2/28 patients had an additional SCID diagnosis of bulimia nervosa, 4/28 of panic disorder, 6/28 suffered with generalised anxiety disorder, 2/28 with social phobia, 2/28

with dysthymia, 1/28 with obsessive compulsive disorder, 1/28 with dissociative disorder and 3/28 post traumatic stress disorder. The total number of patients that had a comorbid diagnosis was 15/28.

The SCID-II self report questionnaire was applied to all subjects to assess axis-II diagnoses. A total of 19/28 patients reported features compatible with a significant axis-II psychopathology. Of these, 14/28 suffered with avoidant, 10/28 with obsessive compulsive, 4/28 with schizoid, 2/28 with schizotypal, 2/28 with paranoid, 10/28 with depressive, 5/28 with dependent, 4/28 with borderline, and 2/28 with narcissistic personality disorder features (multiple conditions in some patients). Of the 21 patients followed up, 15/21 had significant self-reported axis-II psychopathology, suggesting no differential follow up of those with or without comorbid axis-II psychopathology.

There was no difference in the proportion of patients with or without comorbid axis-II psychopathology between subsequent treatment responders (5/14 with and 9/14 without) and non-responders (6/14 with and 8/14 without; $\chi^2=0.143$, $p=0.71$).

The mean \pm SD Cortisol/DHEA ratio on admission between patients with and without features of axis-II psychopathology did not differ and was 29.4 \pm 45.6 for patients with and 40.7 \pm 27.6 for patients without any comorbid axis-II features ($t=.682$, d.f.=26, $p=0.50$). Mean \pm SD Cortisol/DHEA ratio on discharge was 28.4 \pm 25.2 for patients with and 39.0 \pm 28.3 for patients without any comorbid axis-II features ($t=-.863$, d.f.=18, $p=0.40$). Mean \pm SD DHEA on admission was 27.4 \pm 17.5 nmol/l in patients with and 16.2 \pm 11.4 nmol/l in patients without any comorbid axis-II features ($t=1.748$, d.f.=26, $p=0.09$), and mean \pm SD DHEA on discharge was 23.8 \pm 17.9 nmol/l in patients with and 12.1 \pm 6.8 nmol/l in patients without any comorbid axis-II features ($t=.863$, d.f.=18,

$p=0.40$). Mean cortisol on admission was 431.8 ± 144.4 nmol/l in patients with and 454.9 ± 202.2 nmol/l in patients without any comorbid axis-II features ($t=.347$, d.f.=26, $p=0.73$), and on discharge it was 378.2 ± 109.8 nmol/l and 353.6 ± 130.3 nmol/l respectively ($t=.449$, d.f.=18, $p=0.65$).

Re-assessment post treatment

All 28 patients were re-assessed clinically after a period of individually-optimised inpatient treatment including psychopharmacotherapy based on the Maudsley Prescribing Guidelines 8th edition (Taylor et al., 2005), although blood measures could only be repeated in 21/28 subjects. Patients participating in the study remained on antidepressants and mood stabilisers which were not withdrawn in accord with ethical practice. At the time of first testing, 3/28 patients were medication free. Of those medicated, 24/28 were taking various classes of antidepressants, 19/28 were taking one or more mood stabilisers, 13/28 were taking antipsychotics, 5/28 were taking thyroid supplementation, 11/28 were taking benzodiazepines and 1/28 was taking buspirone.

At the time of the second testing – post-intervention – all subjects were on medication. All 21/21 were on various classes of antidepressants, 14/21 were taking one or more mood stabilisers, 10/21 were taking antipsychotics, 8/21 were taking thyroid supplementation, 4/21 were taking buspirone and 3/21 were taking benzodiazepines.

On discharge the number of patients was 21. However for one of them there was insufficient volume to do the analyses for both cortisol and DHEA, hence only the DHEA assay was performed. Therefore, on discharge we have 20 cortisol values (9 for responders and 11 for non responders). This explains the mild fluctuation in d.f. The

numbers for both patients for whom there are values on admission and for controls are 68 (28 patients and 40 controls).

Comparisons of treatment resistant depressed patients and controls

Values of cortisol, DHEA and the Cortisol/DHEA ratio are shown in Table 1. Cortisol levels were significantly higher in patients than controls ($t=3.23$, d.f.=66, $p=0.002$), but DHEA levels did not differ ($t=-0.52$, d.f.=66, $p=0.61$). The ratio of Cortisol/DHEA was significantly elevated in patients (on log-transformed data, $t=2.10$, d.f.=66, $p=0.04$).

Effect of inpatient treatment

The changes in cortisol and DHEA measures between admission and discharge in the subgroup of 21 patients is shown in Table 1. Cortisol levels were significantly lower after intervention ($t=3.020$, d.f.=19, $p=0.007$), but DHEA ($t=1.229$, d.f.=20, $p=0.23$) and the ratio of Cortisol/DHEA ($t=.163$, d.f.=19, $p=0.87$ on log transformed data) were unchanged after treatment.

Table 1. Cortisol (nmol/l), DHEA (nmol/l) and the Cortisol/DHEA ratio in patients with Treatment Resistant Unipolar Depression (n=28) and healthy controls (n=40) and in patients with Treatment Resistant Unipolar Depression before and after inpatient therapy (n=21). Values are shown as means±standard deviations.

	<i>Patients (n=28)</i>	<i>Controls (n=40)</i>	<i>P value</i>	<i>Patients pre treatment (21 patients)</i>	<i>patients post treatment (21 patients)</i>	<i>P value</i>
Cortisol (nmol/l)	439±162	322.0±136	<0.001	466.7±150	369.9±114	<0.001
DHEA (nmol/l)	23.8±16.5	26.7±26.0	0.60	23.9±16.1	19.9±15.9	0.23
Cort/DHEA Ratio	33.0±40.5 ^a	20.8±24 ^a	0.04	36.9±46.8 ^a	32.1±26.1 ^a	0.87
HAM-D	25±5.1	-	-	23±6.0	16±8.8	0.05

Key: DHEA (dehydroepiandrosterone); BMI (body mass index); MMSE (Mini-Mental State Examination); BAI (Beck Anxiety Inventory); PSQI (Pittsburgh Sleep Quality Index); RLCQ (Recent Life Changes Questionnaire); HAM-D (Hamilton Depression Rating Scale)

^a non-parametric data

Comparison of responders and non-responders to treatment

Cortisol, DHEA and their ratio were compared following treatment between responders and non-responders (see Table 2).

There was no difference in cortisol level between responders and non-responders either before ($t=.015$, d.f.=26, $p=0.98$) or after treatment ($t=.492$, d.f.=18, $p=0.63$). In contrast, baseline DHEA levels were lower in those who went on to respond to treatment ($t=-2.163$, d.f.=26, $p=0.04$), although this difference was no longer significant at the time of discharge ($t=-1.517$, d.f.=19, $p=0.15$). Baseline Cortisol/DHEA ratio was higher in

those who went on to respond to treatment (on log transformed data $t=2.533$, d.f.= 26, $p=0.02$) and remained higher on discharge ($t=2.175$, d.f.=18, $p=0.05$).

A within group comparison comparing pre-treatment and post treatment values separately for responders ($n=9$) and non-responders ($n=11$) (inadequate volume for analysis for 1 patient) showed that there was a trend for cortisol values to be lower after treatment in both responders (mean fall -98.3 (163.4) nmol/l, $t=1.99$, d.f.=8, $p=0.07$) and non-responders (mean fall -95.6 (125.5) nmol/l, $t=2.287$, d.f.=10, $p=0.05$). In responders, there were no significant changes in DHEA (mean fall -3.76 (13.6) nmol/l, $t=.916$, d.f.=9, $p=0.38$) or the Cortisol/DHEA ratio (mean fall -10.1 (42.3), $t=.452$, d.f.=8, $p=0.66$). Similarly, there were no changes in these values in non-responders (DHEA mean fall -4.2 (16.8) nmol/l, $t=.794$, d.f.=10, $p=0.45$; Cortisol/DHEA mean increase 1.5 (5.0) nmol/l, $t=-.622$, d.f.=8, $p=0.55$). Looked at another way, the mean change in the Cortisol/DHEA ratio did not differ significantly between responders and non-responders ($t=-.812$, d.f.=18, $p=0.43$).

Table 2. Cortisol (nmol/l), DHEA (nmol/l) and Cortisol/DHEA ratios in patients judged to be responders (n=14) and non-responders (n=14) to inpatient treatment. Values are shown as means±standard deviations.

	<i>Responders n=14</i>	<i>Non responders n=14</i>	<i>P value</i>
Cortisol admission (nmol/l)	439±201.7	440±116.5	0.98
Cortisol discharge^a (nmol/l)	381±122.1	355±110	0.63
DHEA admission (nmol/l)	17.5±15.0	30.1±15.9	0.04
DHEA discharge^a (nmol/l)	15.0±12.3	25.3±18.3	0.15
Cort/DHEA admission	47.5±53.5 ^b	18.5±9.4 ^b	0.01
Cort/DHEA discharge^a	41.9±31.0	20.1±11.1	0.05
HAM-D admission	25±5.6	25±4.8	0.79
HAM-D discharge^a	9.4±4.9	22.7±6.5	<.00

Effect of Medication

Medication was examined both in responders and non-responders at the time of the first testing and after the inpatient treatment intervention, at the time of the second testing.

There were no notable differences at the time of first testing. The only notable differences on the post-intervention testing were that more responders than non-responders were taking lamotrigine, (but not because they had failed to respond to lithium) (8/14 responders and 3/14 non-responders), whereas more non-responders than

responders were taking lithium (3/14 responders and 8/14 non responders). In addition, there was no difference in the level of prior treatment-resistance in responders and non-responders. 10/14 of the responders and 13/14 of the non responders had received ECT.

The effect of medication on hormone results at baseline was examined. No differences were found between patients who were or were not taking lamotrigine in cortisol ($t=0.69$, $d.f.=26$, $p=0.50$), DHEA ($t=1.46$, $d.f.=26$, $p=0.16$) or the Cortisol/DHEA ratio ($t=0.07$, $d.f.=26$, $p=0.95$). Similarly, we found no effect of lithium on cortisol ($t=1.28$, $d.f.=26$, $p=0.21$), DHEA ($t=2.0$, $d.f.=26$, $p=0.06$) and the Cortisol/DHEA ratio ($t=1.14$, $d.f.=26$, $p=0.16$). Taking thyroid supplements (cortisol: $t=0.55$, $d.f.=26$, $p=0.59$; DHEA: $t=0.04$, $d.f.=26$, $p=0.97$; Cortisol/DHEA ratio: $t=0.76$, $d.f.=26$, $p=0.46$) or an antipsychotic (cortisol: $t=1.7$, $d.f.=26$, $p=0.10$; DHEA: $t=0.87$, $d.f.=26$, $p=0.40$; Cortisol/DHEA ratio $t=0.71$, $d.f.=26$, $p=0.49$) did not significantly alter hormone values.

Other variables

At baseline, no differences were found between patients who were smoking and not smoking for cortisol ($t=0.09$, $d.f.=26$, $p=0.93$), DHEA ($t=0.97$, $d.f.=26$, $p=0.34$) and the Cortisol/DHEA ratio ($t=1.14$, $d.f.=26$, $p=0.27$). There was no difference between female patients who were pre and post menopausal on cortisol ($t=1.34$, $d.f.=20$, $p=0.20$), DHEA ($t=0.31$, $d.f.=20$, $p=0.76$) and Cortisol/DHEA ratio ($t=0.56$, $d.f.=20$, $p=0.58$) and no difference between male and female patients (cortisol: $t=1.26$, $d.f.=26$, $p=0.22$; DHEA: $t=0.42$, $d.f.=26$, $p=0.68$; Cortisol/DHEA ratio: $t=0.23$, $d.f.=26$, $p=0.82$). There

was no effect of the presence of physical illness on cortisol ($t=1.85$, d.f.= 26, $p=0.08$), DHEA ($t=1.8$, d.f.=26, $p=0.09$) or the Cortisol/DHEA ratio $t=.36$, d.f.=26, $p=0.72$).

In controls, there was lower cortisol in females (males 377 nmol/l, females 286 nmol/l; $t=2.16$, d.f.=38, $p=0.04$) but not for DHEA ($t=0.048$, d.f.=38, $p=0.96$) or the Cortisol/DHEA ratio ($t=0.351$, d.f.=38, $p=0.72$).

There was a negative correlation between cortisol and BMI in controls ($r= -0.40$, $p=0.01$), but not in patients ($r = 0.13$, $p = 0.58$). There was no correlation between BMI and DHEA or the Cortisol/DHEA ratio.

Regarding age we found the expected inverse correlation between DHEA and age as expected in controls ($r=-0.43$, $p<0.001$). This was not present in patients ($r=-0.20$, $p=0.30$). There was no link between cortisol or the Cortisol/DHEA ratio and age in patients or controls.

Stability of Cortisol/DHEA ratio from 0900 hrs to 1700 hrs

Table 3 shows the values at the five time points across the day in the sub-group of healthy controls. A repeated measures analysis of variance was performed separately for Cortisol, DHEA and the Cortisol/DHEA ratio. Results for cortisol showed a significant effect of time ($F_{(2.86, 56)}=2.907$, $p=0.05$). However, there was no significant effect of time for DHEA ($F_{(1.261, 56)}=2.366$, $p=0.13$) or for the Cortisol/DHEA ratio ($F_{(3.414, 56)}=1.042$, $p=0.39$). Thus, although there was an identifiable diurnal variation seen in

cortisol, DHEA covaried with the cortisol in a similar manner, such that there was no significant change in the cortisol/DHEA ratio during the time that was measured.

Table 3. Cortisol (nmol/l), DHEA (nmol/l) and the cortisol/DHEA ratio from 0900-1700 h in healthy controls (n=15). Values are shown as means±standard deviations.

	<i>0900 h</i>	<i>1100 h</i>	<i>1300 h</i>	<i>1500 h</i>	<i>1700 h</i>
Cortisol (nmol/l)	379.7±186	219.7±65	316.2±146	247.0±128	271.7±179
DHEA (nmol/l)	34.3±38.7	20.5±13.6	22.0±11.9	23.7±14.7	22.4±14.1
Cortisol/DHEA	19.1±14	16.5±12.8	18.7±11.7	14.4±11.5	17.7±17.7

STUDY II and III, TREATMENT RESISTANT UNIPOLAR AND BIPOLAR DEPRESSION /SALIVA

Design

Subjects

Treatment Resistant Unipolar Depression

37 subjects with TRUD and a mean \pm SD HAM-D 21 Item score of 23.5 \pm 5.5, were recruited in the study. 21/37 of these undertook the test whilst at home and 16/37 subjects undertook the test during their current inpatient stay in the ADU.

17/37 subjects were recruited as part of a 5 year longitudinal study. Patients who have been inpatients on the ward in the last ten years were invited to come back to the ward for an assessment. During this assessment a battery of tests was administered including the HAM-D 21 Item. Patients were invited to take part in the saliva study. A salivary kit was given to them with clear instructions on salivary collection and they were either invited to come back to the ward to return the pack, or to post the pack. A small reimbursement was administered to all patients who took part in the study.

Although at the time of their recruitment all of these subjects had only outpatient follow up, all of them had previously been admitted to the ADU at least on one occasion, in the previous 5 years. Their admission usually lasted several months.

4/37 subjects were recruited from the Outpatient Affective Disorders Clinic.

8/37 patients were tested in the summer, 10/37 in autumn, 9/37 in the winter and 9/37 in the spring (missing data on 1 patient). Patients were matched for gender, age and body mass index (BMI) with healthy controls.

Treatment Resistant Unipolar Depression in remission

21 subjects with TRUD in remission and a mean \pm SD HAM-D 21 Item of 5.4 ± 3.3 were recruited in the study. Of these 12/21 subjects were recruited as part of the same longitudinal study as described above. These patients had been previously inpatients on the ward (ADU), during the previous 5 years.

6/21 subjects were recruited when they reached the stage of remission, whilst still ward inpatients, or immediately following their discharge. Remission was defined as a HAM-D 21 Item score of 10 or less for more than 2 weeks.

3/21 patients were recruited from the Outpatient Affective Disorders Clinic.

Of the subjects recruited in this group, 8/21 repeated the test during both the acute phase of their illness and during remission.

Treatment Resistant Bipolar Depression

17 subjects with TRBD and a mean \pm SD HAM-D 21 Item of 23.9 ± 4.5 were recruited into the study. 7/17 of these subjects were recruited during their inpatient stay in ADU and 10/17 subjects completed the test from home.

4/17 of these were previously inpatients at the ADU and were recruited as part of the 5 year longitudinal study described above. 3/17 of them were consequently re-admitted to the ADU.

4/17 subjects were recruited from the Outpatient Affective Disorders Clinic.

Treatment Resistant Bipolar Depression in remission

20 subjects with TRBD in remission and a mean \pm SD HAM-D 21 Item of 4.0 \pm 2.9 were recruited in this study. 10/20 subjects were recruited as part of the same 5 year longitudinal study as described above and had previously been inpatients on the ward or completed the test immediately prior to their discharge. The rest of the patients (10/20) were recruited from the Outpatient Affective Disorders Clinic and completed the test from home.

Duration of illness

Duration of illness (median(IQR)) since the age of onset was 13 (19) years, in subjects with TRUD. It was 14 (12) years in subjects with TRUD, 15 (13) years in subjects with TRBD and 22 (14) years in subjects with TRBD in remission. Regarding the duration of illness there were no statistically significant differences between groups with Unipolar and Bipolar Depression both during the active and remitted phase of their illness.

The median (IQR) duration of the current depressive episode was 4 (5) years in patients with TRUD and 3 (3) years in patients with TRBD. There were no differences between the 2 groups with Unipolar and Bipolar Depression regarding their duration of episode. There were also no statistically significant differences in the age of onset of depression

between groups (age of onset for subjects with Unipolar Depression 29 (12) years and 28 (14) years for subjects with Bipolar Depression), or between groups in remission.

Procedures

Discussed in Chapter 2.

Hormone Assays

Discussed in chapter 2.

Analysis

Discussed in chapter 2.

Results

Subject characteristics

Treatment Resistant Unipolar Depression

There were no statistically significant differences between patients and controls in age ($t=0.6$, d.f.=79, $p=0.55$), or gender ($\chi^2=0.591$, d.f.=1, $p=0.49$). Patients had a higher BMI 30.0 ± 7.3 compared to controls BMI 26.3 ± 4.5 , $p=0.01$.

Treatment Resistant Unipolar Depression in remission

There were no statistically significant differences between patients and controls in age ($t=-1.1$, d.f.=47, $p=0.17$), BMI ($t=34.8$, d.f.=25, $p=0.09$), or gender ($\chi^2=2.8$, d.f.=1, $p=0.14$).

There were also no statistically significant differences between patients with TRUD and those in remission in terms of age ($t=-.767$, d.f.=51, $p=0.44$), BMI ($t=.896$, d.f.=53, $p=0.37$), or gender ($\chi^2=1.13$, d.f.=1, $p=0.34$).

Treatment Resistant Bipolar Depression

There were no statistically significant differences between patients and controls in age ($t=-.589$, d.f.=63, $p=0.56$), BMI ($t=-1.4$, d.f.=29, $p=0.17$), or gender ($\chi^2=.485$, d.f.=1, $p=0.55$). Furthermore there were no statistically significant differences between patients with TRUD and TRBD in age ($t=-.063$, d.f.=51, $p=0.95$), BMI ($t=1.193$, d.f.=50, $p=0.24$), or gender ($\chi^2=1.215$, d.f.=1, $p=0.35$).

Treatment Resistant Bipolar Depression in remission

There were no significant differences between patients and controls in age ($t=-1.4$, d.f.=47, $p=0.17$), BMI ($t=33.2$, d.f.=26, $p=0.15$), or gender ($\chi^2=0.1$, d.f.=1, $p=1.0$). Furthermore there were no statistically significant differences between subjects with TRUD and TRBD in remission with regards to age ($t=-.107$, d.f.=37, $p=0.92$), BMI ($t=.649$, d.f.=35, $p=2.32$), or gender ($\chi^2=1.476$, d.f.=1, $p=0.28$).

Controls

The same group and number of controls were used for all comparisons.

Comparison of patients with Treatment Resistant Unipolar Depression and controls

CAR

AUC_g

The CAR (AUC_g) was higher in patients compared to controls both on Day 1 ($t=2.317$, $d.f.=73$, $p=0.02$, 31 patients 1392.2 ± 560.0 nmol/l.min vs 44 controls 1126.0 ± 434.9 nmol/l.min) and Day 2 ($t=2.307$, $d.f.=70$, $p=0.02$, 32 patients 1339.5 ± 550.6 nmol/l.min vs 40 controls 1073.7 ± 427.2 nmol/l.min), and when the mean of the 2 days was calculated ($t=2.576$, $d.f.=64$, $p=0.01$, 27 patients 1385.6 ± 516.0 nmol/l.min vs 39 controls 1097.7 ± 392.0 nmol/l.min).

AUC_i

The AUC_i was not different in patients compared to controls on Day 1 ($t=.125$, $d.f.=73$, $p=0.90$, 31 patients 144.9 ± 660.2 nmol/l.min vs 44 controls 128.0 ± 509.5 nmol/l.min), or Day 2 ($t=1.811$, $d.f.=70$, $p=0.07$, 32 patients 307.9 ± 529.6 nmol/l.min vs 40 controls 74.9 ± 552.2 nmol/l.min), and when the mean of the 2 days was calculated ($t=1.084$, $d.f.=64$, $p=0.28$, 27 patients 232.6 ± 468.9 nmol/l.min vs 39 controls 108.6 ± 448.9 nmol/l.min).

Salivary cortisol concentrations at 0, 15, 30, 45, 60 and 90 minutes post awakening

Single cortisol values (concentrations) were compared between groups. Cortisol was not different post awakening on Day 1 ($t=1.523$, $d.f.=77$, $p=0.13$), or Day 2 ($t=.384$, $d.f.=78$, $p=0.70$). Similarly cortisol was not different at 15 minutes following awakening on Day 1 ($t=1.550$, $d.f.=79$, $p=0.12$), or Day 2 ($t=1.518$, $d.f.=77$, $p=0.13$), or at 30 minutes

following awakening on Day 1 ($t=1.178$, $d.f.=79$, $p=0.24$), or on Day 2 ($t=1.818$, $d.f.=76$, $p=0.07$). At 45 minutes following awakening cortisol was higher on Day 1 in patients compared to controls ($t=2.475$, $d.f.=79$, $p=0.01$), but not on Day 2 ($t=1.596$, $d.f.=77$, $p=0.11$). Similarly at 60 minutes following awakening cortisol was higher on Day 1 in patients compared to controls ($t=2.427$, $d.f.=78$, $p=0.02$), but not on Day 2 ($t=1.758$, $d.f.=76$, $p=0.08$). At 90 minutes following awakening cortisol was higher in patients compared to controls on Day 1 ($t=3.863$, $d.f.=78$, $p<0.001$), and also on Day 2 ($t=1.988$, $d.f.=73$, $p=0.05$).

Salivary cortisol concentrations post awakening are shown in Table 4 and Figures 12 and 13.

Table 4. Salivary cortisol concentrations (nmol/l) in patients with Treatment Resistant Unipolar Depression and healthy controls (*: statistically significant data).

<i>Cortisol</i>						
Day 1 (no of subjects)	Awakening	+15 mins	+30 mins	+45 mins	+60 mins	+90 mins
Patients (34-35)	13.4±8.4	15.8±6.5	17.2±7.3	17.7±7.3*	15.3±7.7*	13.6±7.2*
Controls (45-46)	11.0±5.8	13.8±5.4	15.4±6.6	13.9±6.5*	11.6±5.8*	8.8±3.8*
Day 2 (no of subjects)						
Patients (34-37)	12.1±7.3	15.2±6.2	17.3±7.8	15.7±7.5	14.2±7.0	12.5±7.4*
Controls (41-45)	11.5±5.9	13.2±5.5	14.3±6.6	13.1±6.8	11.6±6.1	9.6±5.4*

Cortisol during the day (AUCg)

Secretion of cortisol during the day (AUCg) was higher in patients on Day 1 ($t=2.317$, d.f.=73, $p=0.02$, 31 patients 98.0 ± 48.3 nmol/l.h vs 44 controls 75.7 ± 31.4 nmol/l.h), Day 2 ($z=-3.300$, $p<0.001$, 30 patients median (IQR) 90.6 (39.6) nmol/l.h vs 40 controls median (IQR) 66.5 (27.3) nmol/l.h), Day 3 ($z=-2.859$, d.f.=63, $p<0.001$, 25 patients median (IQR) 95.0 (49.4) nmol/l.h vs 40 controls median (IQR) 67.5 (22.6) nmol/l.h), but not on Day 4 ($t=1.195$, d.f.=61, $p=0.24$, 25 patients 88.5 ± 32.7 nmol/l.h vs 38 controls 78.8 ± 30.8 nmol/l.h) (Figures 14 and 15).

Concentration of cortisol at noon was higher in patients compared to controls on Day 2 and 3. At 10 pm cortisol was higher in patients, only on Day 1. On awakening cortisol did not differ between groups.

Salivary cortisol concentrations in the morning, noon and evening are shown in Table 5.

Table 5. Cortisol concentrations (nmol/l) in patients with Treatment Resistant Unipolar Depression and healthy controls in the morning, noon and evening over 4 days. All data are given as means±standard deviations, except for those indicated with a symbol ^a (medians (IQR)). An independent t-test was used for comparisons and a Mann-Whitney U test for non parametric data (indicated by the symbol ^a).

<i>Cortisol</i>						
Day 1 (no of subjects)	awakening	p value	noon	p value	10pm	p value
Patients (33-34)	13.4±8.4		5.8±5.8 ^a		2.4±2.2 ^a	
Controls (43-46)	11.0±5.8	ns	4.4±2.9 ^a	ns ^a	1.7±1.7 ^a	0.02 ^a
Day 2 (no of subjects)						
Patients (33-35)	10.9±7.7 ^a		6.7±4.9 ^a		2.2±1.9 ^a	
Controls (42-45)	10.0±5.9 ^a	ns ^a	3.5±1.8 ^a	0.000 ^a	1.8±1.4 ^a	0.08 ^a
Day 3 (no of subjects)						
Patients (29-33)	11.7±5.1		5.4±3.9 ^a		2.3±2.8 ^a	
Controls (42-44)	11.3±4.4	ns	4.1±2.1 ^a	0.02 ^a	1.7±1.8 ^a	ns ^a
Day 4 (no of subjects)						
Patients (28-34)	12.7±7.0		4.9±3.4 ^a		2.4±3.2 ^a	
Controls (39-43)	11.1±5.4	0.08	4.1±2.2 ^a	0.09 ^a	2.4±2.9 ^a	ns ^a

DHEA

Single salivary concentrations of DHEA at 0-90 minutes post awakening did not differ between patients and controls.

Values (concentrations) of DHEA are shown in Table 6 and Table 7.

Table 6. DHEA concentrations (nmol/l) in patients with Treatment Resistant Unipolar Depression and healthy controls. All data are given as means±standard deviations, except for those indicated with a symbol ^a (medians (IQR)).

DHEA						
Day 1 (no of subjects)	Awakening	+15 mins	+30 mins	+45 mins	+60 mins	+90 mins
Patients (29-31)	1.1±1.4 ^a	1.0±1.2 ^a	1.1±0.8	0.8±0.8 ^a	0.9±0.6	0.6±0.7 ^a
Controls (40-43)	1.4±1.7 ^a	1.2±1.4 ^a	1.2±0.8	0.8±0.8 ^a	0.7±0.5	0.6±0.4 ^a
Day 2 (no of subjects)						
Patients (32-34)	1.1±1.6 ^a	1.0±1.2 ^a	1.0±0.6	1.0±0.6	0.8±0.7 ^a	0.7±0.9 ^a
Controls (39-43)	1.5±1.5 ^a	1.2±1.2 ^a	1.3±0.8	1.1±0.8	0.9±1.0 ^a	0.6±0.8 ^a

Table 7. DHEA concentrations (nmol/l) in patients with Treatment Resistant Unipolar Depression and healthy controls in the morning, noon and evening over 4 days. All data are given as medians (IQR). A Mann-Whitney U test for non parametric data (indicated by the symbol ^a).

DHEA						
Day 1 (no of subjects)	awakening	p value	noon	p value	10pm	p value
Patients (29-32)	1.1±1.4 ^a		0.6±0.7 ^a		0.4±0.5 ^a	
Controls (39-42)	1.3±1.7 ^a	ns ^a	0.5±0.6 ^a	ns ^a	0.3±1.3 ^a	ns ^a
Day 2 (no of subjects)						
Patients (31-33)	1.1±1.6 ^a		0.6±0.6 ^a		0.4±0.5 ^a	
Controls (37-41)	1.5±1.5 ^a	ns ^a	0.5±0.5 ^a	ns ^a	0.3±0.3 ^a	ns ^a
Day 3 (no of subjects)						
Patients (25-29)	1.4±1.5 ^a		0.6±0.5 ^a		0.3±0.2 ^a	
Controls (35-38)	1.5±1.4 ^a	ns ^a	0.4±0.3 ^a	ns ^a	0.3±0.4 ^a	ns ^a
Day 4 (no of subjects)						
Patients (21-26)	1.2±1.1 ^a		0.6±0.8 ^a		0.3±0.4 ^a	
Controls (34-37)	1.6±1.8 ^a	ns ^a	0.5±0.4 ^a	ns ^a	0.3±0.4 ^a	ns ^a

DHEA secretion post awakening (AUCg)

DHEA secretion in the morning following awakening (DHEA response to awakening) did not differ between subjects (Day 1, $t=1.173$, d.f.= 63, $p=0.86$, 27 patients 87.9 ± 53.7 nmol/l.min vs 37 controls 85.7 ± 46.4 nmol/l.min; Day 2, $t=-1.538$, d.f.=64, $p=0.13$, 29 patients 84.9 ± 47.1 nmol/l.min vs 37 controls 109.7 ± 75.9 nmol/l.min).

DHEA secretion during the day (AUCg)

Daily secretion of DHEA (AUCg) did not differ between subjects with TRUD and controls (Day 1, $z=-.405$, $p=0.69$, 27 patients median (IQR) 9.7 (9.6) nmol/l.h vs 35 controls 8.3 (5.8) nmol/l.h; Day 2, $z=-.141$, $p=0.88$, 30 patients median (IQR) 10.0 (8.8) nmol/l.h vs 34 controls 8.5 (8.4) nmol/l.h; Day 3, $z=-.222$, $p=0.82$; Day 4, $z=-1.107$, $p=0.27$).

Cortisol/DHEA ratio

The ratio of the Cortisol/DHEA post awakening is shown in Table 8.

Table 8. Cortisol/DHEA ratio in patients with Treatment Resistant Unipolar Depression and healthy controls. All data are given as means \pm standard deviations, except for those indicated with a symbol ^a (medians (IQR)).

<i>Cortisol/DHEA</i>						
Day 1 (no of subjects)	Awakening	+15 mins	+30 mins	+45 mins	+60 mins	+90 mins
Patients (28-31)	8.9 \pm 12.6 ^a	12.0 \pm 14.6 ^a	17.4 \pm 19.3 ^a	15.9 \pm 24.3 ^a	17.2 \pm 17.9 ^a	14.5 \pm 14.1 ^a
Controls (40-43)	6.6 \pm 6.2 ^a	10.8 \pm 7.6 ^a	14.8 \pm 13.6 ^a	18.5 \pm 13.4 ^a	16.2 \pm 14.4 ^a	14.4 \pm 15.7 ^a
Day 2 (no of subjects)						
Patients (32-34)	8.2 \pm 10.5 ^a	13.2 \pm 17.8 ^a	18.2 \pm 19.4 ^a	16.5 \pm 20.3 ^a	23.2 \pm 17.1	19.3 \pm 17.9 ^a
Controls (38-42)	6.3 \pm 7.6 ^a	9.6 \pm 12.0 ^a	12.0 \pm 12.0 ^a	12.7 \pm 13.1 ^a	18.3 \pm 16.1	13.1 \pm 14.6 ^a

The Cortisol/DHEA ratio during the 4 days, at morning, noon and evening is described at Table 9.

Table 9. Cortisol/DHEA in patients with Treatment Resistant Unipolar Depression and healthy controls in the morning, noon and evening over 4 days. All data are given as medians (IQR). A Mann-Whitney U test for non parametric data (indicated by the symbol ^a).

<i>Cortisol/DHEA</i>						
Day 1 (no of subjects)	awakening	p value	noon	p value	10pm	p value
Patients (29-32)	8.9±12.6 ^a		9.3±13.5 ^a		5.0±10.4 ^a	
Controls (39-42)	6.6±6.2 ^a	0.04 ^a	9.1±5.9 ^a	ns ^a	5.8±6.2 ^a	ns ^a
Day 2 (no of subjects)						
Patients (31-33)	8.2±10.5 ^a		12.2±19.4 ^a		6.5±7.3 ^a	
Controls (37-41)	6.2±7.6 ^a	ns ^a	8.6±7.8 ^a	ns ^a	6.1±9.2 ^a	ns ^a
Day 3 (no of subjects)						
Patients (25-29)	11.1±9.7 ^a		8.0±13.5 ^a		5.5±8.1 ^a	
Controls (35-38)	7.1±6.1 ^a	ns ^a	9.2±7.2 ^a	ns ^a	5.8±7.1 ^a	ns ^a
Day 4 (no of subjects)						
Patients (21-26)	10.9±10.3 ^a		7.4±13.2 ^a		7.1±9.7 ^a	
Controls (34-37)	7.2±9.5 ^a	ns ^a	10.7±13.3 ^a	ns ^a	6.6±6.8 ^a	ns ^a

Cortisol/DHEA ratio (AUCg/DHEA secretion) post awakening

The ratio of Cortisol/DHEA measured by dividing the CAR (AUCg) by the output of DHEA following awakening was not different between patients and controls on Day 1 ($z=-1.173$, $p=0.24$, 26 patients median (IQR) 14.7 (16.8) vs 37 controls 14.5 (9.3)), but was higher in patients compared to controls on Day 2 ($z=-2.061$, $p=0.04$, 28 patients median (IQR) 17.3 (21.2) vs 35 controls 11.4 (9.3)).

Cortisol/DHEA ratio (AUCg/DHEA secretion) during the day

Similarly Cortisol/DHEA output during the day measured by dividing the total output of cortisol and that of DHEA during the day (AUCg) did not differ between subjects with TRUD and controls on Day 1 ($z=-.895$, $p=0.37$, 26 patients median (IQR) 156.1 (201.9) vs 34 controls 125.2 (77.6)), but was higher on Day 2 ($t=2.069$, $d.f.=35$, $p=0.05$, 28 patients 195.8 ± 146.7 vs 33 controls 134.2 ± 62.4). It was not different on Day 3 ($z=-.410$, $p=0.68$), or Day 4 ($z=-.952$, $p=0.34$).

Comparison of patients with Treatment Resistant Unipolar Depression in remission and controls

CAR

AUCg

The CAR (AUCg) was not different in patients compared to controls on Day 1 ($t=-.977$, $d.f.=61$, $p=0.32$, 19 patients 1002.2 ± 518.7 nmol/l.min vs 44 controls 1126.0 ± 434.9 nmol/l.min), Day 2 ($t=-.618$, $d.f.=56$, $p=0.54$, 18 patients 996.8 ± 461.8 nmol/l.min vs 40 controls 1073.7 ± 427.2 nmol/l.min) and when the mean of the 2 days was calculated ($t=-.698$, $d.f.=53$, $p=0.49$, 16 patients 1011.2 ± 476.4 nmol/l.min vs 39 controls 1097.7 ± 392.0 nmol/l.min).

AUCi

The AUCi was not different in patients compared to controls on Day 1 ($t=-1.004$, $d.f.=61$, $p=0.32$, 19 patients -8.6 ± 460.0 nmol/l.min vs 44 controls 128.0 ± 509.5 nmol/l.min), Day 2 ($z=-.252$, $p=0.80$, 18 patients median (IQR) 139.9 (120.9) nmol/l.min vs 40 controls median (IQR) 140.6 (669.0) nmol/l.min) and when the mean

of the 2 days was calculated ($t=-.240$, d.f.=53, $p=0.81$, 16 patients 78.9 ± 328.2 nmol/l.min vs 39 controls 108.6 ± 448.9 nmol/l.min).

Salivary cortisol concentrations at 0, 15, 30, 45, 60 and 90 minutes post awakening

Single cortisol values following awakening did not differ between groups on Day 1 ($t=-1.136$, d.f.=65, $p=0.89$), or Day 2 ($t=-1.215$, d.f.=62, $p=0.23$). Similarly there was no difference in cortisol at 15 minutes following awakening on Day 1 ($t=-1.506$, d.f.=65, $p=0.14$), or Day 2 ($t=-1.388$, d.f.=61, $p=0.17$), at 30 minutes following awakening on Day 1 ($t=-1.745$, d.f.=65, $p=0.09$), or on Day 2 ($t=-.366$, d.f.=60, $p=0.71$), at 45 minutes following awakening on Day 1 ($t=-1.357$, d.f.=64, $p=0.18$), or Day 2 ($t=-.289$, d.f.=59, $p=0.77$), at 60 minutes following awakening on Day 1 ($t=-.797$, d.f.=64, $p=0.43$), or on Day 2 ($t=-.230$, d.f.=59, $p=0.82$) and at 90 minutes following awakening on Day 1 ($t=.448$, d.f.=62, $p=0.66$), or on Day 2 ($t=-.474$, d.f.=57, $p=0.64$).

Single salivary cortisol concentrations post awakening are shown in Table 10 and Figures 16 and 17.

Table 10. Cortisol concentrations (nmol/l) in patients with remitted Treatment Resistant Unipolar Depression and healthy controls. All data are given as means±standard deviations, except for those indicated with a symbol ^a (medians (IQR)).

<i>Cortisol</i>						
Day 1 (no of subjects)	Awakening	+15 mins	+30 mins	+45 mins	+60 mins	+90 mins
Patients (19-21)	10.8±5.1	11.5±6.3	12.2±7.3	11.5±6.8	10.3±6.1	9.3±6.2
Controls (45-46)	11.0±5.8	13.8±5.4	15.4±6.6	13.9±6.5	11.6±5.8	8.8±3.8
Day 2 (no of subjects)						
Patients (18-19)	10.5±9.0 ^a	10.9±6.9	13.6±7.6	12.6±6.5	12.2±4.9	8.9±3.7
Controls (41-45)	10,0±5.9 ^a	13.2±5.5	14.3±6.6	13.1±6.8	11.6±6.1	9.6±5.4

Secretion of cortisol during the day (AUCg)

Secretion of cortisol (AUCg) was not different between patients and controls (Day 1, $t=.408$, d.f.=60, $p=0.68$, 19 patients 78.9 ± 19.2 nmol/l.h vs 43 controls 75.7 ± 31.4 nmol/l.h; Day 2, $z=-.798$, $p=0.42$, 16 patients median (IQR) 68.4 (38.6) nmol/l.h vs 40 controls median (IQR) 66.5 (27.3) nmol/l.h; Day 3, $z=-.869$, $p=0.36$, 15 patients median (IQR) 76.0 (35.0) nmol/l.h vs 40 controls 67.5 (22.5) nmol/l.h; Day 4, $t=.793$, d.f.=51, $p=0.43$, 15 patients 85.8 ± 24.1 nmol/l.h vs 38 controls 78.8 ± 30.8 nmol/l.h).

Single values of cortisol in the morning, noon and evening are shown in Table 11 and Figures 18 and 19.

Table 11. Cortisol concentrations (nmol/l) in patients with Treatment Resistant Unipolar Depression in remission and healthy controls in the morning, noon and evening over 4 days. All data are given as means±standard deviations, except for those indicated with a symbol ^a (medians (IQR)). An independent t-test was used for comparisons and a Mann-Whitney U test for non parametric data (indicated by the symbol ^a).

<i>Cortisol</i>						
Day 1 (no of subjects)	awakening	p value	noon	p value	10pm	p value
Patients (19-21)	10.8±5.1		5.5±3.2 ^a		2.6±2.3 ^a	
Controls (43-46)	11.0±5.8	ns	4.4±2.9 ^a	ns ^a	1.7±1.7 ^a	ns ^a
Day 2 (no of subjects)						
Patients (18-19)	10.5±9.0 ^a		4.5±2.8 ^a		1.9±1.8 ^a	
Controls (42-45)	10.0±5.9 ^a	ns ^a	3.5±1.8 ^a	ns ^a	1.8±1.4 ^a	ns ^a
Day 3 (no of subjects)						
Patients (16-20)	10.3±4.8		5.2±2.1 ^a		2.2±1.7 ^a	
Controls (42-44)	11.3±4.4	ns	4.1±2.1 ^a	ns ^a	1.6±1.7 ^a	ns ^a
Day 4 (no of subjects)						
Patients (15-19)	12.1±7.1 ^a		5.5±3.4 ^a		2.5±1.6 ^a	
Controls (39-43)	9.8±6.3 ^a	ns ^a	4.1±2.2 ^a	ns ^a	2.3±2.9 ^a	ns ^a

DHEA

Single salivary concentrations of DHEA (0-90 minutes) post awakening did not differ between patients and controls on days 1 and 2 (Table 12).

Table 12. DHEA concentrations (nmol/l) in patients with remitted Treatment Resistant Unipolar Depression and healthy controls. All data are given as means±standard deviations, except for those indicated with a symbol ^a (medians (IQR)).

DHEA						
Day 1 (no of subjects)	Awakening	+15 mins	+30 mins	+45 mins	+60 mins	+90 mins
Patients (16-18)	0.8±2.9 ^a	0.9±2.2 ^a	0.7±1.2 ^a	1.1±1.2	0.5±1.5 ^a	0.6±0.5
Controls (40-43)	1.4±1.7 ^a	1.2±1.4 ^a	0.9±1.1 ^a	0.9±0.8	0.6±0.5 ^a	0.7±0.5
Day 2 (no of subjects)						
Patients (14-15)	1.2±4.2 ^a	1.1±1.3 ^a	0.7±1.3 ^a	0.6±1.1 ^a	0.6±1.0 ^a	0.7±0.9 ^a
Controls (39-43)	1.5±1.5 ^a	1.2±1.2 ^a	1.1±1.1 ^a	0.9±0.9 ^a	0.9±1.0 ^a	0.6±0.8 ^a

DHEA secretion post awakening (AUCg)

DHEA secretion in the morning following awakening (DHEA response to awakening) did not differ between subjects (Day 1, $t=.173$, d.f.= 63, $p=0.86$, 27 patients 87.9 ± 53.7 nmol/l.min vs 37 controls 85.7 ± 46.4 nmol/l.min; Day 2, $t=-1.538$, d.f.=64, $p=0.13$, 29 patients 84.9 ± 47.1 nmol/l.min vs 37 controls 109.7 ± 75.9 nmol/l.min).

DHEA secretion during the day (AUCg)

Daily secretion of DHEA did not differ between patients and controls (Day 1, $z=-1.404$, $p=0.16$, 13 patients median (IQR) 6.1 (8.5) nmol/l.h vs 35 controls 8.3 (5.8) nmol/l.h; Day 2, $t=-.300$, d.f.=43, $p=0.76$, 11 patients 10.2 ± 8.9 nmol/l.h vs 34 controls 11.0 ± 7.9 nmol/l.h; Day 3, $z=-.583$, $p=0.56$; Day 4, $z=-.185$, $p=0.85$).

Daily concentrations of DHEA are shown in Table 13.

Table 13. DHEA concentrations (nmol/l) in patients with Treatment Resistant Unipolar Depression in remission and healthy controls in the morning, noon and evening over 4 days. All data are given as medians (IQR). A Mann-Whitney U test was used for non parametric data (indicated by the symbol ^a).

DHEA						
Day 1 (no of subjects)	awakening	p value	noon	p value	10pm	p value
Patients (16-18)	0.8±2.9 ^a		0.5±0.6 ^a		0.3±0.6 ^a	
Controls (39-42)	1.3±1.7 ^a	ns ^a	0.5±0.6 ^a	ns ^a	0.3±1.3 ^a	ns ^a
Day 2 (no of subjects)						
Patients (14-15)	1.2±4.1 ^a		0.3±0.5 ^a		0.3±0.4 ^a	
Controls (37-41)	1.5±1.5 ^a	ns ^a	0.5±0.5 ^a	ns ^a	0.3±0.3 ^a	ns ^a
Day 3 (no of subjects)						
Patients (13-17)	1.2±3.1 ^a		0.9±1.1 ^a		0.3±0.6 ^a	
Controls (35-38)	1.5±1.4 ^a	ns ^a	0.4±0.3 ^a	ns ^a	0.3±0.4 ^a	ns ^a
Day 4 (no of subjects)						
Patients (15)	1.2±1.4 ^a		0.7±1.1 ^a		0.4±0.7 ^a	
Controls (34-37)	1.6±1.8 ^a	ns ^a	0.5±0.4 ^a	ns ^a	0.3±0.4 ^a	ns ^a

Cortisol/DHEA ratio

Values of Cortisol/DHEA are shown in Table 14 and 15. The ratio was lower in patients at noon on Day 3.

Table 14. Cortisol/DHEA ratio in patients with Treatment Resistant Unipolar Depression in remission and healthy controls. All data are given as medians (IQR)^a.

<i>Cortisol/DHEA</i>						
Day 1 (no of subjects)	Awakening	+15 mins	+30 mins	+45 mins	+60 mins	+90 mins
Patients (15-18)	13.0±20.1 ^a	12.1±20.1 ^a	18.3±25.6 ^a	21.2±26.3 ^a	22.2±47.4 ^a	16.0±40.7 ^a
Controls (40-43)	6.6±6.2 ^a	10.8±7.6 ^a	14.8±13.6 ^a	18.5±13.4 ^a	16.2±14.4 ^a	14.4±15.7 ^a
Day 2 (no of subjects)						
Patients (14-15)	6.5±15.3 ^a	11.1±22.4 ^a	21.4±26.5 ^a	16.9±31.7 ^a	20.6±33.1 ^a	16.3±37.1 ^a
Controls (38-42)	6.3±7.6 ^a	9.6±12.0 ^a	12.0±12.0 ^a	12.7±13.1 ^a	14.3±16.5 ^a	13.1±14.6 ^a

Table 15. Cortisol/DHEA ratio in patients with Treatment Resistant Unipolar Depression in remission and healthy controls in the morning, noon and evening over 4 days. All data are given as medians (IQR). A Mann-Whitney U test was used for non parametric data (indicated by the symbol ^a).

<i>Cortisol/DHEA</i>						
Day 1 (no of subjects)	awakening	p value	noon	p value	10pm	p value
Patients (33-34)	13.0±20.1 ^a		10.9±17.5 ^a		6.3±10.9 ^a	
Controls (43-46)	6.6±6.2 ^a	ns ^a	9.1±5.9 ^a	ns ^a	5.8±6.2 ^a	ns ^a
Day 2 (no of subjects)						
Patients (33-35)	6.5±15.3 ^a		16.2±16.6 ^a		6.5±11.6 ^a	
Controls (42-45)	6.3±7.6 ^a	ns ^a	8.6±7.8 ^a	ns ^a	6.1±9.2 ^a	ns ^a
Day 3 (no of subjects)						
Patients (14-17)	5.5±12.5 ^a		5.2±9.4 ^a		5.7±7.2 ^a	
Controls (33-37)	7.1±6.1 ^a	ns ^a	9.2±7.2 ^a	0.02 ^a	5.8±7.1 ^a	ns ^a
Day 4 (no of subjects)						
Patients (14-15)	10.1±7.3 ^a		6.8±13.7 ^a		6.7±11.2 ^a	
Controls (31-36)	7.2±9.5 ^a	0.08 ^a	10.8±13.2 ^a	0.09 ^a	6.6±6.8 ^a	ns ^a

Cortisol/DHEA ratio (AUCg/DHEA secretion) post awakening

The ratio of Cortisol/DHEA measured by dividing the CAR (AUCg) by the output of DHEA following awakening was not different between patients and controls on Day 1 ($z=-.852$, $p=0.39$, 13 patients median (IQR) 22.8 (30.3) vs 37 controls 14.5 (9.3), or on Day 2 ($t=1.580$, $d.f.=14$, $p=0.13$, 13 patients 20.0±15.0 vs 35 controls 13.1±7.6).

Cortisol/DHEA ratio (AUCg/DHEA secretion) during the day

The ratio of Cortisol/DHEA during the day measured by dividing the total output of cortisol and that of DHEA during the day (AUCg) did not differ between subjects with TRUD and controls on Day 1 ($z=-1.576$, $p=0.11$, 12 patients median (IQR) 195.5 (169.2) vs 34 controls 125.2 (77.6)), Day 2 ($t=1.680$, $d.f.=11$, $p=0.12$, 11 patients 217.7 ± 160.9 vs 33 controls 134.2 ± 62.4), Day 3 ($z=-.501$, $p=0.61$), or Day 4 ($z=-.562$, $p=0.57$).

Comparison of patients with Treatment Resistant Bipolar Depression and controls

CAR

AUCg

The CAR (AUCg) was lower on Day 1 in subjects with TRBD compared to controls ($t=-2.165$, $d.f.=56$, $p=0.03$, 14 patients 852.6 ± 322.5 nmol/l.min vs 44 controls 1126.0 ± 434.9 nmol/l.min). On Day 2 the AUCg did not differ between patients and controls ($z=-.244$, $p=0.80$, 17 patients median (IQR) 960.0 (779.6) nmol/l.min vs 40 controls median (IQR) 1060.4 (682.1) nmol/l.min). When the mean of the 2 days was calculated there was a trend for significance and the AUCg was lower in patients compared to controls ($z=-1.796$, $p=0.07$, 14 patients median (IQR) 836.4 (336.6) nmol/l.min vs 39 controls median (IQR) 1106.2 (619.1) nmol/l.min).

AUCi

The AUCi was lower on Day 1 in subjects with TRBD compared to controls ($z=-2.017$, $p=0.04$, 14 patients median (IQR) -209.2 (434.4) nmol/l.min vs 44 controls median (IQR) 184.5 (652.1) nmol/l.min). On Day 2 the AUCi did not differ between patients

and controls ($t=-.130$, $d.f.=55$, $p=0.90$, 17 patients 53.2 ± 698.0 nmol/l.min vs 40 controls 74.9 ± 552.2 nmol/l.min). When the mean of the 2 days was calculated the AUCi was not significantly different between patients and controls ($t=-1.460$, $d.f.=51$, $p=0.15$, 14 patients -88.8 ± 387.2 nmol/l.min vs 39 controls 108.6 ± 448.9 nmol/l.min).

The AUCi was also calculated after excluding patients that had never been admitted to the ADU and only had outpatient follow up (4 patients in total), as these patients were less severely ill compared to patients with inpatient psychiatric admissions. It was found that in that case the AUCi was lower in patients with TRBD compared to controls on Day 1 ($t=3.907$, $d.f.=51$, $p=0.000$, 11 patients -224.0 ± 156.3 nmol/l.min vs 44 controls 130.0 ± 509.5 nmol/l.min) and as a mean of the 2 days ($t=2.133$, $d.f.=48$, $p=0.04$, 11 patients -202.6 ± 333.7 nmol/l.min vs 44 controls 108.6 ± 448.9). On the contrary, when the AUCg was calculated it did not differ between the 2 groups after excluding the outpatients (Day 1, $t=.399$, $d.f.=53$, $p=0.143$; Day 2, $t=.384$, $d.f.=51$, $p=0.761$).

Salivary cortisol concentrations at 0, 15, 30, 45, 60 and 90 minutes post awakening

Concentration of cortisol on awakening was not different between patients and controls both on Day 1 ($t=-.299$, $d.f.=59$, $p=0.76$), or Day 2 ($z=-.331$, $p=0.74$). Similarly there was no difference between subjects for cortisol concentration at 15 minutes following awakening on Day 1 ($t=-.579$, $d.f.=59$, $p=0.56$), or Day 2 ($t=.260$, $d.f.=59$, $p=0.80$), or at 30 minutes following awakening on Day 1 ($t=-1.192$, $d.f.=59$, $p=0.23$), or on Day 2 ($t=.080$, $d.f.=58$, $p=0.94$). At 45 minutes following awakening cortisol was lower on Day 1 in patients with TRBD compared to controls ($t=-2.287$, $d.f.=58$, $p=0.03$), but it was not lower on Day 2 ($t=-.349$, $d.f.=57$, $p=0.73$). Similarly at 60 minutes following awakening cortisol was lower on Day 1 in patients compared to controls ($t=-2.416$,

d.f.=58, $p=0.02$), but it was not lower on Day 2 ($t=-.185$, d.f.=57, $p=0.85$). At 90 minutes following awakening cortisol was lower in patients compared to controls on Day 1 ($t=-2.407$, d.f.=57, $p=0.02$), but not on Day 2 ($z=-.607$, $p=0.54$).

When the mean of the 2 days was calculated concentration of cortisol was no different between patients and controls at 0 ($t=-.290$, d.f.=57, $p=0.78$, 15 patients 10.8 ± 4.3 nmol/l vs 44 controls 11.2 ± 4.9 nmol/l), 15 ($t=-.404$, d.f.=57, $p=0.69$, 15 patients 13.0 ± 4.4 nmol/l vs 44 controls 13.5 ± 4.7 nmol/l) and 30 minutes following awakening ($t=-.758$, d.f.=56, $p=0.45$, 15 patients 13.5 ± 6.4 nmol/l vs 43 controls 14.8 ± 5.8 nmol/l). Thereafter cortisol was lower in patients compared to controls ie. at 45 minutes ($t=-2.015$, d.f.=54, $p=0.05$, 14 patients 10.1 ± 5.0 nmol/l vs 42 controls 13.5 ± 5.6 nmol/l), 60 minutes ($z=-2.063$, $p=0.04$, 14 patients 8.0 ± 3.3 nmol/l vs 42 controls 11.2 ± 5.0 nmol/l) and 90 minutes following awakening ($t=-2.307$, d.f.=53, $p=0.02$, 14 patients 6.6 ± 2.1 nmol/l vs 41 controls 9.1 ± 3.9 nmol/l).

Single salivary cortisol concentrations post awakening are shown in Table 16 and Figures 12 and 13.

Table 16. Cortisol concentrations (nmol/l) in patients with Treatment Resistant Bipolar Depression and healthy controls. All data are given as means±standard deviations, except for those indicated with a symbol ^a (medians (IQR) (*: statistically significant data)).

<i>Cortisol</i>						
Day 1 (no of subjects)	Awakening	+15 mins	+30 mins	+45 mins	+60 mins	+90 mins
Patients (14-15)	10.5±4.8	12.9±5.3	13.0±17.0	9.7±4.5*	7.7±2.9*	6.2±2.5*
Controls (45-46)	11.0±5.8	13.8±5.4	15.4±6.6	13.9±6.5*	11.6±5.8*	8.8±3.8*
Day 2 (no of subjects)						
Patients (17)	10.9±5.9 ^a	13.6±5.0	14.5±7.9	12.4±8.1	11.9±6.9	7.8±5.7 ^a
Controls (41-45)	10.0±5.9 ^a	13.2±5.5	14.3±6.6	13.1±6.8	11.6±6.1	8.5±5.4 ^a

Secretion of cortisol during the day (AUCg)

Daily secretion of cortisol (AUCg) was not statistically different between patients and controls in 3 of the 4 days (Day 1, $t=-.042$, d.f.=52, $p=0.97$, 11 patients 75.3 ± 17.6 nmol/l.h vs 43 controls 75.7 ± 31.4 nmol/l.h; Day 2, $z=-1.678$, $p=0.09$, 16 patients median (IQR) 62.9 (18.3) nmol/l.h vs 43 controls median (IQR) 66.5 (27.2) nmol/l.h; Day 4, $z=-1.145$, $p=0.25$, 8 patients median (IQR) 60.4 (27.2) nmol/l.h vs 38 controls median (IQR) 70.4 (36.7) nmol/l.h) (Figures 14 and 15).

On Day 3 however, patients had a higher secretion of cortisol compared to controls ($z=-2.859$, $p=0.04$, 8 patients median (IQR) 79.4 (26.9) nmol/l.h vs 40 controls median (IQR) 65.9 (20.5) nmol/l.h).

Single concentrations of cortisol in the morning, noon and evening are shown in Table 17.

Table 17. Cortisol concentrations (nmol/l) in patients with Treatment Resistant Bipolar Depression and healthy controls in the morning, noon and evening over 4 days. All data are given as means±standard deviations, except for those indicated with a symbol ^a (medians (IQR)). An independent t-test was used for comparisons and a Mann-Whitney U test for non parametric data (indicated by the symbol ^a).

<i>Cortisol</i>						
Day 1 (no of subjects)	awakening	p value	noon	p value	10pm	p value
Patients (13-15)	10.5±4.8		4.4±1.9 ^a		1.6±2.9 ^a	
Controls (43-46)	11.0±5.8	ns	4.4±2.9 ^a	ns ^a	1.7±1.7 ^a	ns ^a
Day 2 (no of subjects)						
Patients (15-17)	10.9±5.9 ^a		5.1±3.5 ^a		2.0±2.1 ^a	
Controls (43-46)	10.0±5.9 ^a	ns ^a	3.5±1.8 ^a	0.03 ^a	1.8±1.4 ^a	ns ^a
Day 3 (no of subjects)						
Patients (11-13)	12.6±7.4		5.7±3.3 ^a		2.8±2.4 ^a	
Controls (42-44)	11.2±4.4	ns	4.1±2.1 ^a	0.03 ^a	1.6±1.7 ^a	0.04 ^a
Day 4 (no of subjects)						
Patients (15-17)	10.3±9.2 ^a		4.7±5.1 ^a		1.9±2.8 ^a	
Controls (39-43)	9.8±6.3 ^a	ns ^a	4.1±2.2 ^a	ns ^a	2.3±2.9 ^a	ns ^a

DHEA

Values (concentrations) of DHEA are described at Table 18.

Table 18. DHEA concentrations (nmol/l) in patients with Treatment Resistant Bipolar Depression and healthy controls. All data are given as means±standard deviations, except for those indicated with a symbol ^a (medians (IQR)).

DHEA						
Day 1 (no of subjects)	Awakening	+15 mins	+30 mins	+45 mins	+60 mins	+90 mins
Patients (14)	0.9±1.7 ^a	1.0±1.4 ^a	0.9±0.7	0.7±0.6	0.5±1.0 ^a	0.5±0.4 ^a
Controls (40-43)	1.4±1.7 ^a	1.2±1.4 ^a	1.2±0.8	0.9±0.6	0.6±0.6 ^a	0.6±0.5 ^a
Day 2 (no of subjects)						
Patients (13-16)	2.2±1.7	1.0±1.7 ^a	1.3±1.1	0.9±0.5	0.9±0.8 ^a	0.8±0.8 ^a
Controls (39-43)	2.0±2.0	1.2±1.2 ^a	1.3±0.8	1.1±0.8	0.9±1.0 ^a	0.6±0.8 ^a

Single concentrations of DHEA post awakening (0-90 minutes) did not differ between patients and controls.

DHEA secretion post awakening (AUCg)

DHEA secretion in the morning following awakening (DHEA response to awakening) did not differ between subjects with TRBD and controls (Day 1, $t=-.330$, d.f.=49, $p=0.74$, 14 patients 80.6 ± 57.7 nmol/l.min vs 37 controls 85.7 ± 46.4 nmol/l.min; Day 2, $z=-1.260$, $p=0.21$, 14 patients median (IQR) 89.3 (88.8) nmol/l.min vs 37 controls 93.9 (85.5) nmol/l.min).

DHEA secretion during the day (AUCg)

Daily secretion of DHEA did not differ between subjects with TRBD and controls (Day 1, $z=-.405$, $p=0.69$, 12 patients median (IQR) 9.9 (10.6) nmol/l.h vs 35 controls 8.3 (5.8)

nmol/l.h; Day 2, $z=-.141$, $p=0.89$, 15 patients median (IQR) 12.7 (12.9) nmol/l.h vs 34 controls 8.5 (8.4) nmol/l.h; Day 3, $z=-1.513$, $p=0.13$; Day 4, $z=-.047$, $p=0.96$).

Table 19. DHEA concentrations (nmol/l) in patients with Treatment Resistant Bipolar Depression and healthy controls in the morning, noon and evening over 4 days. All data are given as medians (IQR). A Mann-Whitney U test was used for non parametric data (indicated by the symbol ^a).

DHEA						
Day 1 (no of subjects)	awakening	p value	noon	p value	10pm	p value
Patients (13-15)	0.9±1.7 ^a		0.6±0.9 ^a		0.4±0.8 ^a	
Controls (39-42)	1.3±1.7 ^a	ns ^a	0.5±0.6 ^a	ns ^a	0.3±1.3 ^a	ns ^a
Day 2 (no of subjects)						
Patients (15-16)	1.5±2.2 ^a		0.5±0.6 ^a		0.4±0.4 ^a	
Controls (37-41)	1.5±1.5 ^a	ns ^a	0.5±0.5 ^a	ns ^a	0.3±0.3 ^a	ns ^a
Day 3 (no of subjects)						
Patients (10-12)	1.7±1.6 ^a		0.6±0.5 ^a		0.5±0.6 ^a	
Controls (35-38)	1.5±1.4 ^a	ns ^a	0.4±0.3 ^a	ns ^a	0.3±0.4 ^a	ns ^a
Day 4 (no of subjects)						
Patients (8-10)	1.7±2.1 ^a		0.6±1.0 ^a		0.3±0.3 ^a	
Controls (34-37)	1.6±1.8 ^a	ns ^a	0.5±0.4 ^a	ns ^a	0.3±0.4 ^a	ns ^a

Cortisol/DHEA ratio

Values of Cortisol/DHEA are shown in Table 20 and 21. Single values of the ratio of Cortisol/DHEA post awakening are shown in Table 18. The ratio was lower in patients at noon on Day 3.

Table 20. Cortisol/DHEA ratio in patients with Treatment Resistant Bipolar Depression and healthy controls. All data are given as medians (IQR).

<i>Cortisol/DHEA</i>						
Day 1 (no of subjects)	Awakening	+15 mins	+30 mins	+45 mins	+60 mins	+90 mins
Patients (13)	7.4±13.4 ^a	12.3±16.5 ^a	18.2±19.5 ^a	13.1±26.1 ^a	12.9±15.8 ^a	9.8±18.0 ^a
Controls (40-43)	6.6±6.2 ^a	10.8±7.6 ^a	14.8±13.6 ^a	18.5±13.4 ^a	16.2±14.4 ^a	14.4±15.7 ^a
Day 2 (no of subjects)						
Patients (14-16)	5.4±4.8 ^a	8.5±7.9 ^a	10.7±36.0 ^a	8.9±8.1 ^a	9.0±12.9 ^a	8.1±7.3 ^a
Controls (39-43)	6.3±7.6 ^a	9.6±12.0 ^a	12.0±12.0 ^a	12.7±13.1 ^a	14.3±16.5 ^a	13.1±14.6 ^a

Cortisol/DHEA ratio (AUCg/DHEA secretion) post awakening

The ratio of Cortisol/DHEA measured by dividing the CAR (AUCg) by the output of DHEA following awakening was not different between patients and controls on Day 1 ($z=-1.173$, $p=0.24$, 13 patients median (IQR) 14.0 (12.0) vs 37 controls median (IQR) 14.5 (9.3)), but did differ on Day 2 ($z=-2.061$, $p=0.04$, 14 patients median (IQR) 7.8 (9.1) vs 35 controls 11.4 (9.3)).

Cortisol/DHEA ratio (AUCg/DHEA secretion) during the day

Similarly the ratio of Cortisol/DHEA during the day measured by dividing the total output of cortisol and that of DHEA during the day (AUCg) did not differ between subjects with TRBD and controls on Day 1 ($z=-.895$, $p=0.37$, 10 patients median (IQR) 125.5 (95.4) vs 34 controls 125.2 (77.6), or on Day 2 ($z=-1.114$, $p=0.26$, 14 patients median (IQR) 119.2 (81.6) vs 33 controls 124.6 (94.0), or Day 3 ($z=-1.236$, $p=0.21$), or Day 4 ($z=.570$, $p=0.57$).

Table 21. Cortisol/DHEA in patients with Treatment Resistant Bipolar Depression and healthy controls in the morning, noon and evening over 4 days. All data are given as medians (IQR). A Mann-Whitney U test was used for non parametric data (indicated by the symbol ^a).

<i>Cortisol/DHEA</i>						
Day 1 (no of subjects)	awakening	p value	noon	p value	10pm	p value
Patients (13-11)	7.4±13.4 ^a		11.4±10.7 ^a		7.3±6.0 ^a	
Controls (40-38)	6.6±6.2 ^a	ns ^a	9.1±5.9 ^a	ns ^a	5.8±6.2 ^a	ns ^a
Day 2 (no of subjects)						
Patients (16-15)	5.4±4.8 ^a		8.8±9.8 ^a		4.0±6.4 ^a	
Controls (40-36)	6.6±6.2 ^a	ns ^a	9.1±5.9 ^a	ns ^a	5.8±6.2 ^a	ns ^a
Day 3 (no of subjects)						
Patients (10-11)	6.9±2.9 ^a		8.3±9.6 ^a		5.4±5.2 ^a	
Controls (33-37)	7.1±6.1 ^a	ns ^a	9.2±7.2 ^a	0.02 ^a	5.8±7.1 ^a	ns ^a
Day 4 (no of subjects)						
Patients (8-9)	6.5±8.1 ^a		6.5±4.0 ^a		7.3±5.6 ^a	
Controls (31-36)	7.2±9.5 ^a	ns ^a	10.8±13.2 ^a	ns ^a	6.6±6.8 ^a	ns ^a

Comparison of patients with Treatment Resistant Bipolar Depression in remission and controls

CAR

AUCg

The CAR (AUCg) was not different between patients and controls on Day 1 ($t=1.483$, $d.f.=62$, $p=0.14$, 20 patients 1293.9 ± 383.6 nmol/l.min vs 44 controls 1126.0 ± 434.9 nmol/l.min), Day 2 ($t=.736$, $d.f.=56$, $p=0.46$, 18 patients 1164.8 ± 454.8 nmol/l.min vs 40 controls 1073.7 ± 427.2 nmol/l.min) and mean of 2 days ($t=1.207$, $d.f.=55$, $p=0.23$, 18 patients 1224.9 ± 315.5 nmol/l.min vs 39 controls 1097.7 ± 392.0 nmol/l.min).

AUCi

The AUCi was no different in patients with TRBD in remission compared to controls on Day 1 ($t=.468$, $d.f.=62$, $p=0.64$, 20 patients 187.8 ± 382.8 nmol/l.min vs 44 controls 128.0 ± 509.5 nmol/l.min), Day 2 ($t=-.174$, $d.f.=56$, $p=0.86$, 18 patients 47.8 ± 544.7 nmol/l.min vs 40 controls 74.9 ± 552.2 nmol/l.min) and mean of 2 days ($t=.014$, $d.f.=55$, $p=0.99$, 18 patients 110.4 ± 432.2 nmol/l.min vs 39 controls 108.6 ± 448.9 nmol/l.min).

Salivary cortisol concentrations at 0, 15, 30, 45, 60 and 90 minutes post awakening

Cortisol concentrations did not differ at 0 minutes post awakening between groups on Day 1 ($t=.867$, $d.f.=64$, $p=0.39$), or Day 2 ($z=-.175$, $p=0.86$). Cortisol was higher in remitted depressed patients with TRBD at 15 minutes following awakening on Day 1 ($t=2.049$, $d.f.=64$, $p=0.04$), but not on Day 2 ($z=-.171$, $p=0.86$). It did not differ between groups at 30 minutes following awakening (Day 1, $z=-.788$, $p=0.43$; Day 2, $t=-.303$, $d.f.=59$, $p=0.76$), at 45 minutes following awakening (Day 1, $t=.778$, $d.f.=64$, $p=0.44$;

Day 2, $t=.062$, $d.f.=58$, $p=0.95$), at 60 minutes following awakening (Day 1, $z=-1.514$, $p=0.13$; Day 2, $z=-.549$, $p=0.58$) and at 90 minutes following awakening (Day 1, $t=1.889$, $d.f.=63$, $p=0.06$; Day 2, $t=.213$, $d.f.=57$, $p=0.83$).

Single salivary cortisol concentrations post awakening are shown in Table 22 and Figures 16 and 17.

Table 22. Cortisol concentrations (nmol/l) in patients with remitted Treatment Resistant Bipolar Depression and healthy controls. All data are given as means \pm standard deviations, except for those indicated with a symbol ^a medians (IQR).

<i>Cortisol</i>						
Day 1 (no of subjects)	Awakening	+15 mins	+30 mins	+45 mins	+60 mins	+90 mins
Patients (20)	12.3 \pm 5.2	16.8 \pm 5.9	15.5 \pm 10.2 ^a	15.2 \pm 4.9	12.7 \pm 4.4 ^a	10.9 \pm 4.7
Controls (45-46)	11.0 \pm 5.8	13.8 \pm 5.4	15.4 \pm 10.5 ^a	13.9 \pm 6.5	10.4 \pm 5.8 ^a	8.8 \pm 3.8
Day 2 (no of subjects)						
Patients (18)	11.9 \pm 11.4 ^a	15.0 \pm 9.0 ^a	14.9 \pm 7.4	13.2 \pm 5.5	10.8 \pm 8.5 ^a	9.9 \pm 3.5
Controls (41-45)	10.0 \pm 5.9 ^a	13.3 \pm 8.1 ^a	14.3 \pm 6.6	13.1 \pm 6.8	10.9 \pm 8.5 ^a	9.6 \pm 5.4

Cortisol during the day (AUCg)

Secretion of cortisol was higher in patients compared to controls in 2 out of 4 days (Day 1, $t=2.576$, $d.f.=57$, $p=0.01$, 16 patients 98.6 \pm 27.4 nmol/l.h vs 43 controls 75.7 \pm 31.4 nmol/l.h; Day 2, $z=-2.939$, $p=0.00$, 16 patients median (IQR) 86.4 (60.6) nmol/l.h vs 40 controls median (IQR) 66.5 (27.3) nmol/l.h; Day 3, $z=-1.397$, $p=0.16$, 16 patients median (IQR) 84.5 (32.2) nmol/l.h vs 43 controls median (IQR) 67.5 (22.5) nmol/l.h;

Day 4, $t=1.074$, $d.f.=49$, $p=0.29$, 13 patients 89.5 ± 31.3 nmol/l.h vs 38 controls 78.8 ± 30.8 nmol/l.h) (Figures 18 and 19).

Single concentrations of cortisol in the morning, noon and evening are described in Table 23. Cortisol concentrations were higher at noon during Day 1, 2, 3 in patients compared to controls.

Table 23. Cortisol concentrations (nmol/l) in patients with remitted Treatment Resistant Bipolar Depression and healthy controls, in the morning, noon and evening over 4 days. All data are given as means±standard deviations, except for those indicated with a symbol ^a (medians (IQR)). An independent t-test was used for comparisons and a Mann-Whitney U test for non parametric data (indicated by the symbol ^a).

<i>Cortisol</i>						
Day 1 (no of subjects)	awakening	p value	noon	p value	10pm	p value
Patients (13-15)	12.3±5.2		6.5±4.1 ^a		2.2±2.2 ^a	
Controls (43-46)	11.0±5.8	ns	4.4±2.9 ^a	<0.001 ^a	1.7±1.7 ^a	ns ^a
Day 2 (no of subjects)						
Patients (15-17)	11.9±11.4 ^a		5.8±2.8 ^a		2.2±2.6 ^a	
Controls (43-46)	10.0±5.9 ^a	ns ^a	3.5±1.8 ^a	<0.001 ^a	1.8±1.4 ^a	ns ^a
Day 3 (no of subjects)						
Patients (11-13)	13.5±6.9		6.1±3.1 ^a		1.7±1.2 ^a	
Controls (42-44)	11.2±4.4	ns	4.1±2.1 ^a	<0.001 ^a	1.6±1.7 ^a	ns ^a
Day 4 (no of subjects)						
Patients (15-18)	13.0±9.4 ^a		5.6±2.8 ^a		1.8±1.1 ^a	
Controls (39-43)	9.8±6.3 ^a	0.04 ^a	4.1±2.2 ^a	ns ^a	2.3±2.9 ^a	ns ^a

DHEA

Values of DHEA are shown in Table 24 and 25.

Single concentrations of DHEA post awakening (0-90 minutes) did not differ between patients and controls.

Table 24. DHEA concentrations (nmol/l) in patients with remitted Treatment Resistant Bipolar Depression and healthy controls. All data are given as means±standard deviations, except for those indicated with a symbol ^a medians (IQR).

<i>DHEA</i>						
Day 1 (no of subjects)	Awakening	+15 mins	+30 mins	+45 mins	+60 mins	+90 mins
Patients (16-17)	1.0±1.0 ^a	1.4±1.1 ^a	1.2±0.9	1.2±1.1	0.5±1.0 ^a	0.8±0.6
Controls (40-43)	1.3±1.7 ^a	1.2±1.4 ^a	1.2±0.8	0.9±0.6	0.6±0.5 ^a	0.7±0.5
Day 2 (no of subjects)						
Patients (15-17)	1.2±1.0	0.9±1.4 ^a	1.2±1.0	0.5±0.7 ^a	1.1±1.0	0.6±1.0 ^a
Controls (39-43)	2.1±2.0	1.2±1.2 ^a	1.3±0.8	0.9±0.9 ^a	1.0±0.7	0.6±0.8 ^a

DHEA secretion post awakening (AUCg)

DHEA secretion in the morning following awakening (DHEA response to awakening) did not differ between subjects with TRBD in remission and controls (Day 1, $t=.625$, d.f.= 31, $p=0.53$, 16 patients 95.5 ± 64.5 nmol/l.min vs 37 controls 85.7 ± 46.4 nmol/l.min; Day 2, $z=-1.499$, $p=0.13$, 14 patients median (IQR) 235.3 (241.2) nmol/l.min vs 37 controls 125.27 (7.6) nmol/l.min).

DHEA secretion during the day (AUCg)

Daily secretion of DHEA (AUCg) did not differ between subjects with remitted TRBD and controls (Day 1, $t=.225$, d.f.=46, $p=0.82$, 13 patients 9.8 ± 7.6 nmol/l.h vs 35 controls 9.3 ± 5.2 nmol/l.h; Day 2, $z=-1.251$, $p=0.21$, 12 patients median (IQR) 6.3 (9.9) nmol/l.h vs 34 controls 8.5 (8.4) nmol/l.h; Day 3, $z=.353$, $p=0.72$; Day 4, $z=-1.200$, $p=0.23$).

Table 25. DHEA concentrations (nmol/l) in patients with Treatment Resistant Bipolar Depression in remission and healthy controls in the morning, noon and evening over 4 days. All data are given as medians (IQR). A Mann-Whitney U test was used for non parametric data (indicated by the symbol ^a).

<i>DHEA</i>						
Day 1 (no of subjects)	awakening	p value	noon	p value	10pm	p value
Patients (29-32)	1.0±1.0 ^a		0.5±0.4 ^a		1.1±1.6 ^a	
Controls (39-42)	1.3±1.7 ^a	ns ^a	0.5±0.6 ^a	ns ^a	0.3±1.3 ^a	ns ^a
Day 2 (no of subjects)						
Patients (31-33)	0.8±0.8 ^a		0.7±1.2 ^a		0.2±0.2 ^a	
Controls (37-41)	1.5±1.5 ^a	ns ^a	0.5±0.5 ^a	ns ^a	0.3±0.3 ^a	ns ^a
Day 3 (no of subjects)						
Patients (25-29)	1.2±2.1 ^a		0.7±0.6 ^a		0.2±0.4 ^a	
Controls (35-38)	1.5±1.4 ^a	ns ^a	0.4±0.3 ^a	ns ^a	0.3±0.4 ^a	ns ^a
Day 4 (no of subjects)						
Patients (21-26)	0.6±1.0 ^a		0.6±0.4 ^a		0.4±0.2 ^a	
Controls (34-37)	1.6±1.8 ^a	0.01 ^a	0.5±0.4 ^a	ns ^a	0.3±0.4 ^a	ns ^a

Cortisol/DHEA ratio

Single values of the ratio of Cortisol/DHEA post awakening are shown in Table 26.

Cortisol/DHEA values during the day (morning, noon, evening) are shown in Table 27.

The ratio was higher in the morning on Day 2 and 4 and at noon on Day 1.

Table 26. Cortisol/DHEA ratio in patients with remitted Treatment Resistant Bipolar Depression and healthy controls. All data are given as medians (IQR).

<i>Cortisol/DHEA</i>						
Day 1 (no of subjects)	Awakening	+15 mins	+30 mins	+45 mins	+60 mins	+90 mins
Patients (16-17)	10.5±7.9 ^a	14.6±19.3 ^a	21.2±33.3 ^a	18.4±31.5 ^a	18.2±37.7 ^a	15.5±20.2 ^a
Controls (40-43)	6.6±6.2 ^a	10.8±7.6 ^a	14.8±13.6 ^a	18.5±13.4 ^a	16.2±14.4 ^a	14.4±15.7 ^a
Day 2 (no of subjects)						
Patients (14-15)	6.5±15.3 ^a	11.1±22.4 ^a	21.4±26.5 ^a	16.9±31.7 ^a	20.6±33.1 ^a	16.3±37.1 ^a
Controls (38-42)	6.3±7.6 ^a	9.6±12.0 ^a	12.0±12.0 ^a	12.7±13.1 ^a	14.3±16.5 ^a	13.1±14.6 ^a

Table 27. Cortisol/DHEA in patients with Treatment Resistant Bipolar Depression in remission and healthy controls in the morning, noon and evening over 4 days. All data are given as medians (IQR). A Mann-Whitney U test was used for non parametric data (indicated by the symbol ^a).

<i>Cortisol/DHEA</i>						
Day 1 (no of subjects)	awakening	p value	noon	p value	10pm	p value
Patients (15-14)	13.0±20.1 ^a		10.9±17.5 ^a		6.3±10.9 ^a	
Controls (38-40)	6.6±6.2 ^a	ns ^a	9.1±5.9 ^a	0.02 ^a	5.8±6.2 ^a	ns ^a
Day 2 (no of subjects)						
Patients (14-15)	10.3±17.7 ^a		9.1±18.9 ^a		8.2±7.6 ^a	
Controls (36-40)	6.3±7.6 ^a	0.04 ^a	8.6±7.8 ^a	ns ^a	6.1±9.2 ^a	ns ^a
Day 3 (no of subjects)						
Patients (11-13)	8.1±26.4 ^a		9.6±13.9 ^a		5.7±15.5 ^a	
Controls (33-37)	7.1±6.1 ^a	ns ^a	9.2±7.2 ^a	ns ^a	5.8±7.1 ^a	ns ^a
Day 4 (no of subjects)						
Patients (12-15)	14.5±37.8 ^a		13.8±13.9 ^a		5.4±9.5 ^a	
Controls (31-36)	7.2±9.5 ^a	0.002 ^a	10.8±13.2 ^a	ns ^a	6.6±6.8 ^a	ns ^a

Cortisol/DHEA ratio (AUCg/DHEA secretion) post awakening

The ratio of Cortisol/DHEA measured by dividing the CAR (AUCg) by the output of DHEA following awakening (AUCg) was not different between patients and controls on Day 1 (z=-1.008, p=0.31, 13 patients median (IQR) 15.8 (22.8) vs 37 controls median (IQR) 14.5 (9.3)), but it was higher in patients on Day 2 (t=2.21, d.f.=14, p=0.04, 14 patients 25.2±19.9 vs 35 controls 13.1±7.6).

Cortisol/DHEA ratio (AUCg/DHEA secretion) during the day

Similarly the ratio of Cortisol/DHEA during the day measured by dividing the total output of cortisol and that of DHEA during the day (AUCg) was higher in subjects with TRBD and controls on Day 1 ($z=-2.212$, $p=0.03$, 13 patients median (IQR) 235.3 (241.2) vs 34 controls median (IQR) 125.2 (77.6)) and also on Day 2 ($t=2.228$, $d.f.=12.0$, $p=0.05$, 12 patients 248.0 ± 172.9 vs 33 controls 134.2 ± 62.4 , but not on Day 3 ($z=-.885$, $p=0.38$), or Day 4 ($z=-1.204$, $p=0.23$).

Comparison of patients with Treatment Resistant Unipolar and Bipolar Depression

CAR

AUCg

The CAR (AUCg) was higher in patients with TRUD compared to those with TRBD (Day 1, $t=3.353$, $d.f.=43$, $p=0.002$, 31 patients with TRUD 1392.2 ± 559.4 nmol/l.min vs 14 patients with TRBD 852.6 ± 322.5 nmol/l.min; Day 2, $t=1.461$, $d.f.=47$, $p=0.15$, 32 patients with TRUD 1339.5 ± 550.6 nmol/l.min vs 17 patients with TRBD 1101.5 ± 527.4 nmol/l.min; mean of 2 days $t=3.216$, $d.f.=39$, $p=0.003$, 27 patients with TRUD 1385.6 ± 516.0 nmol/l.min vs 14 patients with TRBD 902.2 ± 304.3 nmol/l.min) (Figure 1 and 2). When only inpatients were compared the AUCg was also found to be lower in TRBD compared to TRUD (Day 1 $t=.145$, $d.f.=37$, $p=0.01$; mean of 2 days, $t=.125$, $d.f.=34$, $p=0.01$).

AUCi

The AUCi was higher in patients with TRUD compared to those with TRBD (Day 1, $z=-2.023$, $p=0.04$, 31 patients with TRUD median (IQR) 77.2 (861.0) nmol/l.min vs 14 patients with TRBD -209.2 (434.4) nmol/l.min; Day 2, $t=1.501$, d.f.=47, $p=0.14$, 32 patients with TRUD 307.9 (529.6) nmol/l.min vs 17 patients with TRBD 53.2 (628.0) nmol/l.min; mean of 2 days $t=2.201$, d.f.=39, $p=0.03$, 27 patients with TRUD 232.6 ± 468.9 nmol/l.min vs 14 patients with TRBD -88.8 ± 387.2 nmol/l.min). When only inpatients were compared the AUCi was found to be lower in TRBD compared to TRUD (Day 1 $t=.010$, d.f.=33, $p=0.01$; mean of 2 days, $t=.123$, d.f.=34, $p=0.01$)

Salivary cortisol concentrations at 0, 15, 30, 45, 60 and 90 minutes post awakening

Cortisol post awakening was not different between groups (Day 1, $z=-.834$, $p=0.40$; Day 2, $z=-.166$, $p=0.86$). Similarly cortisol did not differ at 15 minutes following awakening (Day 1, $t=1.563$, d.f.=48, $p=0.12$; Day 2, $t=.876$, d.f.=50, $p=0.38$), or at 30 minutes following awakening (Day 1, $t=1.883$, d.f.=48 $p=0.06$; Day 2, $t=1.216$, d.f.=50, $p=0.23$). At 45 minutes following awakening cortisol was higher on Day 1 in patients with TRUD compared to those with TRBD ($t=3.820$, d.f.=47, $p=0.000$), but it did not differ on Day 2 ($t=1.461$, d.f.=52, $p=0.15$). Similarly at 60 minutes following awakening cortisol was higher on Day 1 in TRUD compared to TRBD ($t=3.567$, d.f.=46, $p=0.00$), but it did not differ on Day 2 ($t=1.113$, d.f.=51, $p=0.27$) and it was again higher at 90 minutes following awakening on Day 1 ($t=3.758$, d.f.=47, $p=0.000$).

Secretion of cortisol during the day (AUCg)

On Day 1, daily secretion of cortisol was higher in patients with Acute TRUD compared to those with Acute TRBD ($t=2.238$, d.f.=40, $p=0.03$, 31 patients 98.0 ± 48.3 nmol/l.h vs

11 patients 75.3 ± 17.6 nmol/l.h). Daily cortisol secretion did not differ between groups on the rest of the days (Day 2, $t=1.804$, d.f.=44, $p=0.08$, 30 patients 95.2 ± 33.3 nmol/l.h vs 16 patients 78.8 ± 19.3 nmol/l.h; Day 3, $t=.304$ d.f.=31, $p=0.76$, 25 patients 93.5 ± 27.4 nmol/l.h vs 8 patients 90.3 ± 20.1 nmol/l.h; Day 4, $t=-.140$, d.f.=31, $p=0.90$, 25 patients 88.5 ± 32.7 nmol/l.h vs 8 patients 90.5 ± 44.9 nmol/l.h). Once more not all patients agreed to collect cortisol over 4 days and this is the reason why the numbers are less on days 3 and 4.

Cortisol concentrations on Day 1 and 2 are shown in Figures 1 and 2 (post awakening) and Figures 3 and 4 (during the day).

Single values of cortisol in the morning, noon and evening are shown in Table 28.

Table 28. Cortisol concentrations (nmol/l) in patients with Treatment Resistant Unipolar Depression and Treatment Resistant Bipolar Depression. All data are given as means \pm standard deviations, except for those indicated with a symbol ^a (medians (IQR) (*: statistically significant data)).

<i>Cortisol</i>						
Day 1 (no of subjects)	Awakening	+15 mins	+30 mins	+45 mins	+60 mins	+90 mins
TRUD (33-35)	11.2 \pm 11.0 ^a	15.8 \pm 6.5	17.2 \pm 7.3	17.7 \pm 7.3*	15.3 \pm 7.7*	13.6 \pm 7.2*
TRBD (14-15)	11.2 \pm 8.6 ^a	12.9 \pm 5.3	13.0 \pm 17.0	9.7 \pm 4.5*	7.7 \pm 2.9*	6.2 \pm 2.5*
Day 2 (no of subjects)						
TRUD (34-37)	10.9 \pm 7.7 ^a	15.2 \pm 6.2	17.3 \pm 7.8	15.7 \pm 7.5	14.2 \pm 7.0	10.6 \pm 8.4 ^a
TRBD (17)	10.9 \pm 8.5 ^a	13.6 \pm 5.0	14.5 \pm 7.9	12.4 \pm 8.1	11.9 \pm 6.9	7.8 \pm 6.6 ^a

Table 29. Cortisol concentrations (nmol/l) in patients with Treatment Resistant Unipolar Depression and Treatment Resistant Bipolar Depression in the morning, noon and evening over 4 days. All data are given as means±standard deviations, except for those indicated with a symbol ^a (medians (IQR)). An independent t-test was used for comparisons and a Mann-Whitney U test for non parametric data (indicated by the symbol ^a).

<i>Cortisol</i>						
Day 1 (no of subjects)	awakening	p value	noon	p value	10pm	p value
TRUD (33-34)	11.2±11.0 ^a		5.8±5.7 ^a		2.3±2.2 ^a	
TRBD (13-15)	11.2±8.6 ^a	ns ^a	4.4±1.9 ^a	ns ^a	1.6±2.9 ^a	ns ^a
Day 2 (no of subjects)						
TRUD (33-35)	10.9±7.7 ^a		6.7±4.8 ^a		2.2±1.9 ^a	
TRBD (16-17)	10.9±8.5 ^a	ns ^a	5.1±3.5 ^a	ns ^a	2.0±2.1 ^a	ns ^a
Day 3 (no of subjects)						
TRUD (29-33)	10.4±7.1 ^a		5.4±3.9 ^a		2.3±2.8 ^a	
TRBD (12-14)	11.0±13.2 ^a	ns ^a	5.7±3.3 ^a	ns ^a	2.8±2.4 ^a	ns ^a
Day 4 (no of subjects)						
TRUD (28-34)	12.7±7.0		5.9±2.7		2.3±3.1 ^a	
TRBD (12-13)	12.5±5.3	ns	5.7±3.5	ns	1.9±2.8 ^a	ns ^a

DHEA

Single concentrations of DHEA on awakening and at 15, 30, 45, 60, 90 minutes following awakening did not differ between patients.

Values (concentrations) of DHEA during the day (morning, noon and evening) are shown in Table 30.

DHEA secretion post awakening (AUCg)

DHEA secretion in the morning following awakening (DHEA response to awakening) was also similar between the 2 groups (Day 1, $z=-.640$, $p=0.52$; Day 2, $t=-.68$, $d.f.=41$, $p=0.50$).

DHEA secretion during the day (AUCg)

Daily secretion of DHEA did not differ between subjects with Acute TRUD and TRBD (Day 1, $t=-.475$, $d.f.=37$, $p=0.64$; Day 2 $t=-1.336$ $d.f.=43$, $p=0.20$; Day 3, $z=-1.342$, $p=0.18$; Day 4, $z=-.447$, $p=0.65$).

Table 30. DHEA concentrations (nmol/l) in patients with Treatment Resistant Unipolar Depression and Treatment Resistant Bipolar Depression in the morning, noon and evening over 4 days. All data are given as medians (IQR)). A Mann-Whitney U test was used for non parametric data (indicated by the symbol ^a).

<i>DHEA</i>						
Day 1 (no of subjects)	awakening	p value	noon	p value	10pm	p value
TRUD (29-32)	1.1±1.4 ^a		0.6±0.7 ^a		0.4±0.5 ^a	
TRBD (13-15)	0.9±1.7 ^a	ns ^a	0.6±0.9 ^a	ns ^a	0.4±0.8 ^a	ns ^a
Day 2 (no of subjects)						
TRUD (31-33)	1.1±1.6 ^a		0.6±0.6 ^a		0.4±0.5 ^a	
TRBD (15-16)	1.5±2.2 ^a	ns ^a	0.5±0.6 ^a	ns ^a	0.4±0.4 ^a	ns ^a
Day 3 (no of subjects)						
TRUD (25-29)	1.4±1.5 ^a		0.6±0.5 ^a		0.3±0.2 ^a	
TRBD (10-12)	1.7±1.6 ^a	ns ^a	0.6±0.5 ^a	ns ^a	0.5±0.6 ^a	ns ^a
Day 4 (no of subjects)						
TRUD (21-26)	1.2±1.1 ^a		0.6±0.8 ^a		0.3±0.4 ^a	
TRBD (8-10)	1.7±2.1 ^a	ns ^a	0.6±1.0 ^a	ns ^a	0.3±0.3 ^a	ns ^a

Cortisol/DHEA ratio

Values (concentrations) of Cortisol/DHEA during the day are shown in Table 31. The ratio was higher in TRUD compared to TRBD in the morning on Day 2 and 3.

Cortisol/DHEA ratio (AUCg/DHEA secretion) post awakening

The ratio of Cortisol/DHEA ratio measured by dividing the CAR (AUCg) by the output of DHEA following awakening (AUCg) was not different between subjects with TRUD and TRBD on Day 1 ($z=-.894$, $p=0.37$), or on Day 2 ($z=-1.908$, $p=0.06$).

Cortisol/DHEA ratio (AUCg/DHEA secretion) during the day

Similarly the ratio of Cortisol/DHEA during the day measured by dividing the total output of cortisol and that of DHEA during the day (AUCg) did not differ between subjects with TRUD and TRBD on Day 1 ($z=-.353$, $p=0.72$), or on Day 2 ($z=-1.014$, $p=0.31$), Day 3 ($z=-1.305$, $p=0.19$), or Day 4 ($z=-.850$, $p=0.39$).

Table 31. Cortisol/DHEA ratio in patients with Treatment Resistant Unipolar Depression and Treatment Resistant Bipolar Depression in the morning, noon and evening over 4 days. All data are given as medians (IQR). A Mann-Whitney U test was used for non parametric data (indicated by the symbol ^a).

<i>Cortisol/DHEA</i>						
Day 1 (no of subjects)	awakening	p value	noon	p value	10pm	p value
TRUD (28-31)	8.9±12.6 ^a		9.3±13.5 ^a		5.0±10.4 ^a	
TRBD (11-13)	7.4±13.4 ^a	ns ^a	11.4±10.7 ^a	ns ^a	7.3±6.0 ^a	ns ^a
Day 2 (no of subjects)						
TRUD (29-33)	8.2±10.5 ^a		12.2±19.4 ^a		6.5±7.3 ^a	
TRBD (14-16)	5.4±4.8 ^a	0.05 ^a	8.8±9.8 ^a	ns ^a	4.0±6.4 ^a	ns ^a
Day 3 (no of subjects)						
TRUD (24-29)	11.1±9.7 ^a		8.0±13.5 ^a		5.5±8.1 ^a	
TRBD (10-11)	6.9±2.9 ^a	0.02 ^a	8.3±9.6 ^a	ns ^a	5.4±5.2 ^a	ns ^a
Day 4 (no of subjects)						
TRUD (19-26)	11.0±10.3 ^a		7.4±13.2 ^a		7.1±9.7 ^a	
TRBD (8-9)	6.5±8.1 ^a	0.01 ^a	6.5±4.0 ^a	ns ^a	7.3±5.6 ^a	ns ^a

Cortisol following awakening Day 1

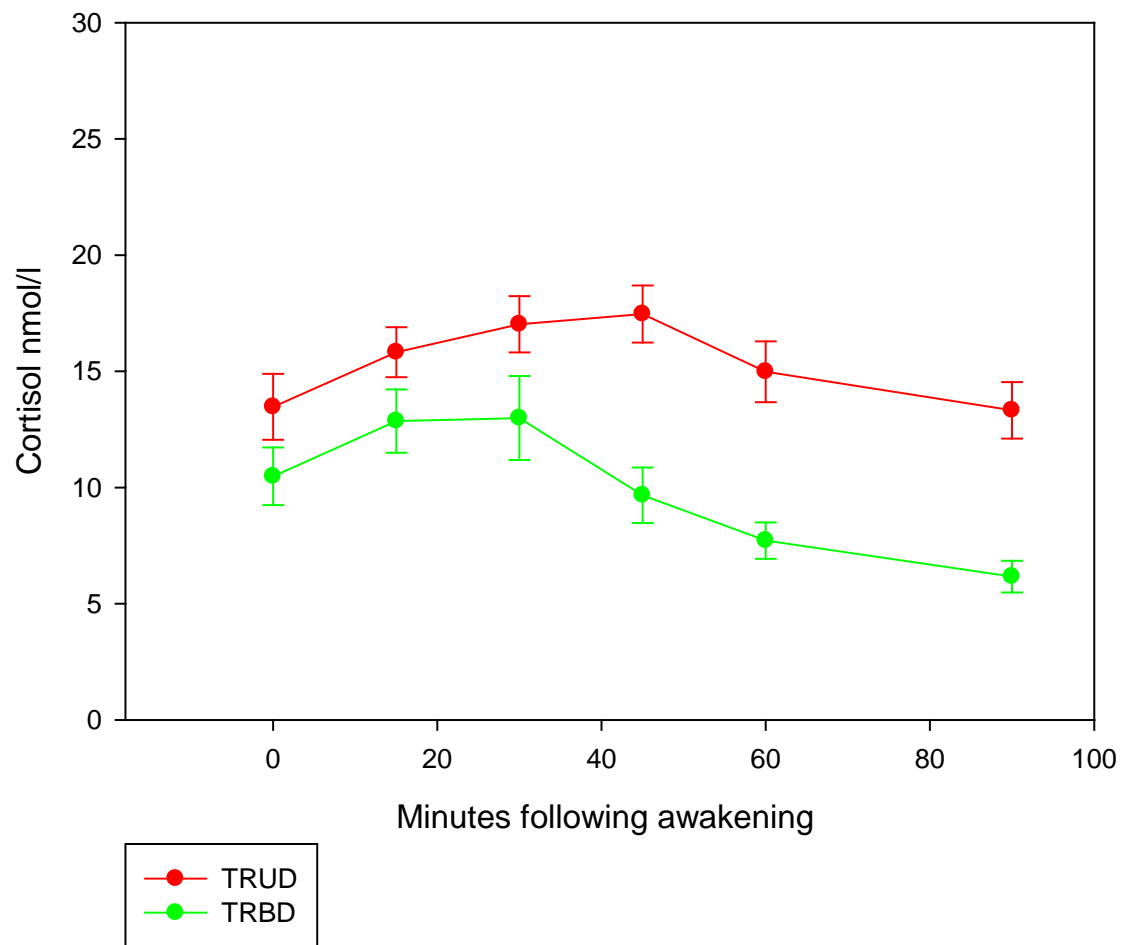


Figure 1. Cortisol concentrations (nmol/l) post awakening 0-90 minutes on Day 1 in 31 patients with Treatment Resistant Unipolar Depression and 14 patients with Treatment Resistant Bipolar Depression.

Cortisol following awakening Day 2

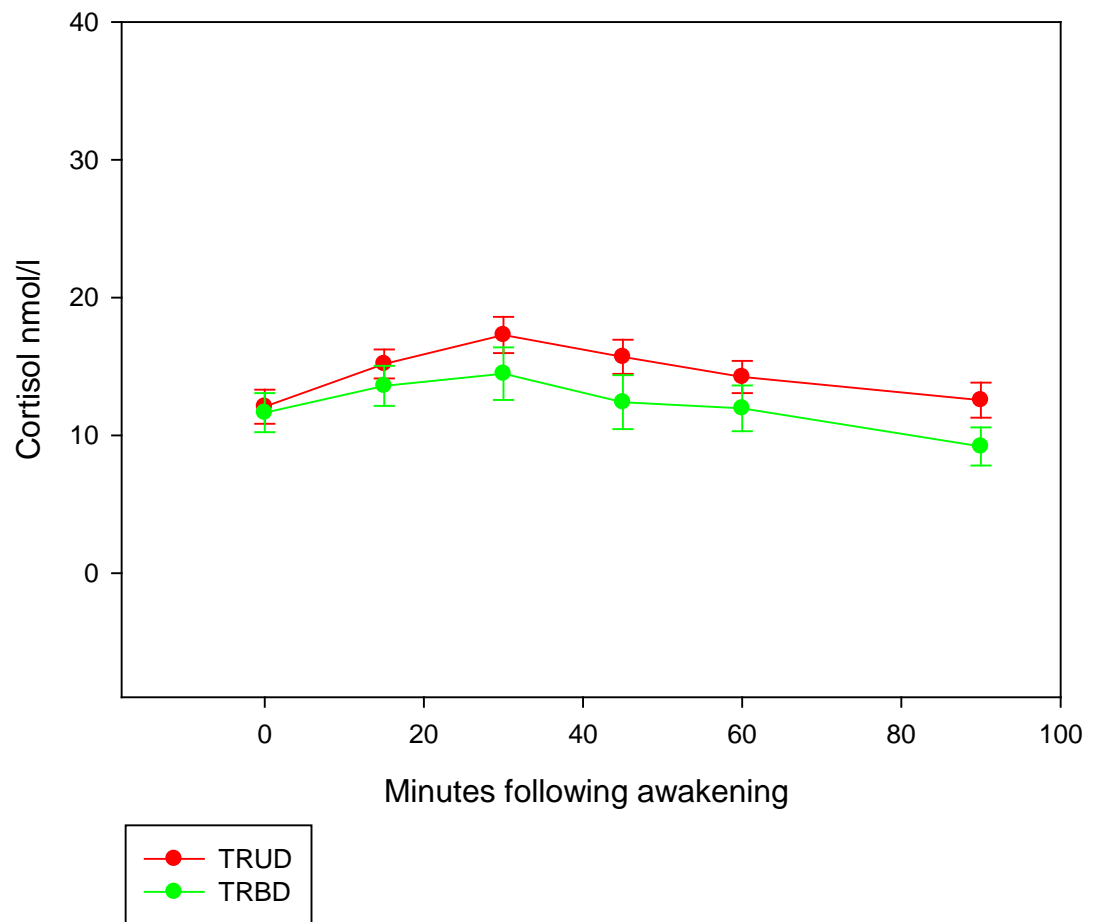


Figure 2. Cortisol concentrations (nmol/l) post awakening 0-90 minutes on Day 1 in 32 patients with Treatment Resistant Unipolar Depression and 17 patients with Treatment Resistant Bipolar Depression.

Cortisol during day Day 1

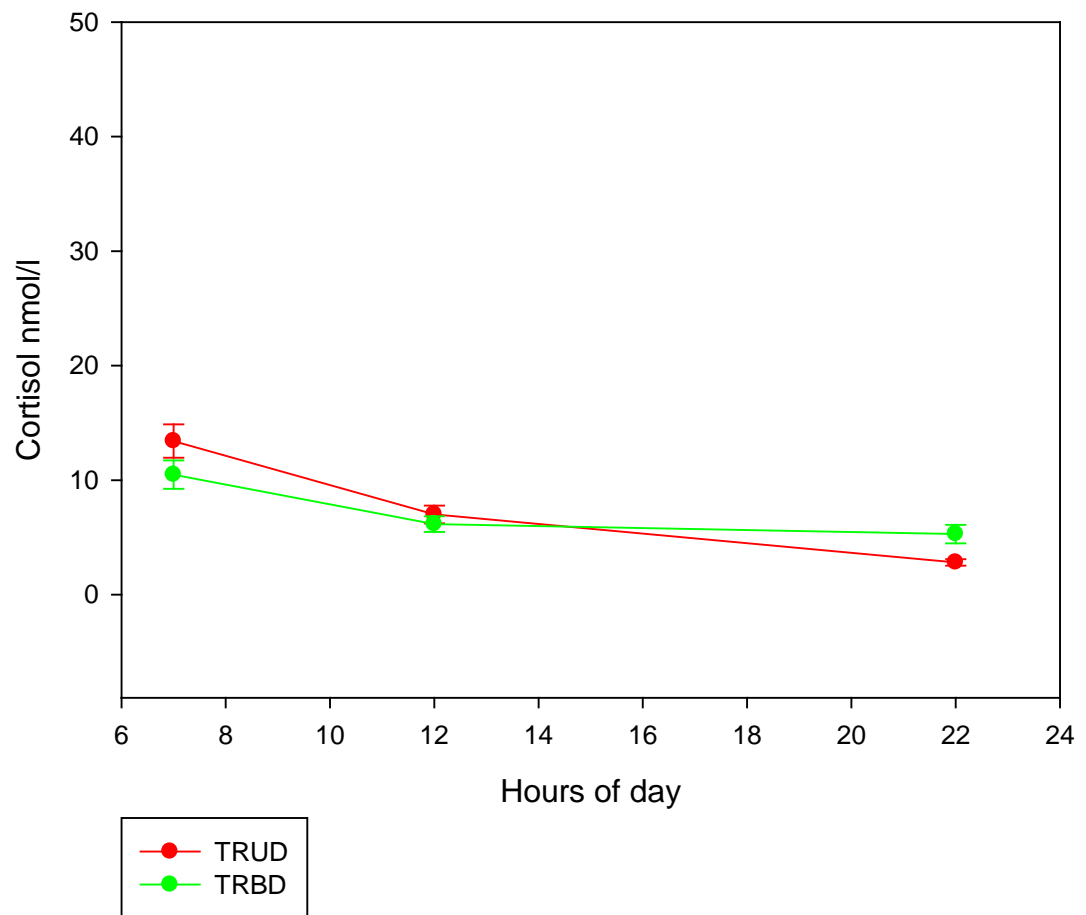


Figure 3. Cortisol concentrations (nmol/l) during the day in 31 patients with Treatment Resistant Unipolar Depression and 11 patients with Treatment Resistant Bipolar Depression.

Cortisol during day Day 2

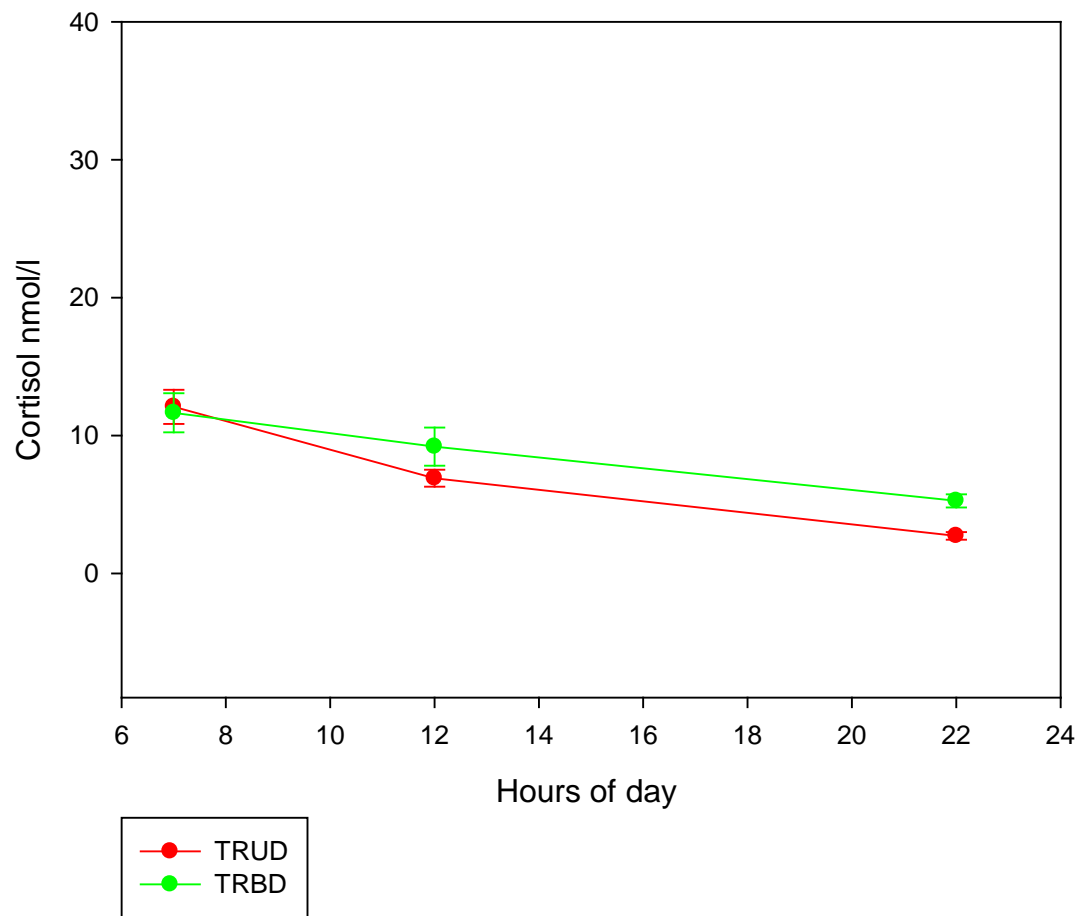


Figure 4. Cortisol concentrations (nmol/l) during the day in 30 patients with Treatment Resistant Unipolar Depression and 16 patients with Treatment Resistant Bipolar Depression on Day 2.

Comparison of patients with Treatment Resistant Unipolar Depression and those in remission

CAR

AUC_g

The CAR (AUC_g) was higher in subjects with TRUD compared those in remission on Day 1 ($t=2.458$, d.f.=48, $p=0.02$, 31 patients 1392.2 ± 559.4 nmol/l.min vs 19 patients 1002.2 ± 518.7 nmol/l.min), Day 2 ($t=2.233$, df=48, $p=0.03$, 32 patients 1339.5 ± 550.6 nmol/l.min vs 18 patients 996.8 ± 461.8 nmol/l.min) and once the mean of the 2 days was calculated ($t=2.365$, d.f.=41, $p=0.02$, 27 patients 1385.6 ± 516.0 nmol/l.min vs 16 patients 1011.2 ± 476.4 nmol/l.min).

AUC_i

The AUC_i was not different in subjects with TRUD compared to those in remission on Day 1 ($t=.889$, d.f.=48, $p=0.38$, 31 patients 144.9 ± 660.2 nmol/l.min vs 19 patients 8.6 ± 460.0 nmol/l.min), Day 2 ($z=-.930$, $p=0.35$, 32 patients median (IQR) 253.9 (596.8) nmol/l.min vs 18 patients median (IQR) 139.9 (120.9) nmol/l.min) and once the mean of the 2 days was calculated ($t=1.153$, d.f.=41, $p=0.26$, 27 patients 232.6 ± 468.9 nmol/l.min vs 16 patients 78.9 ± 328.2 nmol/l.min).

Salivary cortisol concentrations at 0, 15, 30, 45, 60 and 90 minutes post awakening

There was no difference between groups for cortisol values measured on awakening both on Day 1 ($z=-.807$, $p=0.41$), or Day 2 ($z=-.960$, $p=0.34$). At 15 minutes cortisol was higher in patients with TRUD compared to those in remission both on Day 1 ($t=2.428$, d.f.=54, $p=0.02$) and Day 2 ($t=2.328$, d.f.=52, $p=0.02$). At 30 minutes

following awakening cortisol was higher in patients with TRUD compared to those in remission on Day 1 ($t=2.458$, d.f.=54, $p=0.02$), but not on Day 2 ($t=1.667$, d.f.=52, $p=0.10$). Similarly, at 45 minutes following awakening cortisol was higher in patients with TRUD compared to those in remission on Day 1 ($t=3.089$, d.f.=53, $p=0.000$), but not on Day 2 ($t=1.531$, d.f.=54, $p=0.13$). It was also higher in patients with TRUD at 60 minutes following awakening on Day 1 ($t=2.441$, d.f.=52, $p=0.02$), but not on Day 2 ($t=1.654$, d.f.=53, $p=0.10$) and at 90 minutes following awakening on Day 1 ($t=2.179$, d.f.=52, $p=0.03$), but not on Day 2 ($t=1.936$, d.f.=50, $p=0.06$).

Single cortisol concentrations following awakening are shown in Table 32 and Figures 5 and 6.

Table 32. Cortisol concentrations (nmol/l) in patients with Treatment Resistant Unipolar Depression and those in remission. All data are given as means±standard deviations, except for those indicated with a symbol ^a (medians±IQR) (*: statistically significant data).

<i>Cortisol</i>						
Day 1 (no of subjects)	Awakening	+15 mins	+30 mins	+45 mins	+60 mins	+90 mins
Patients acute (34-35)	11.2±11.0 ^a	15.8±6.5*	17.2±7.3*	17.7±7.3*	15.3±7.7*	13.6±7.2*
Patients remission (19-21)	10.6±9.3 ^a	11.5±6.3*	12.2±7.3*	11.5±6.8*	10.4±6.1*	9.3±6.2*
Day 2 (no of subjects)						
Patients acute (34-37)	10.9±7.7 ^a	15.2±6.2*	17.3±7.8	15.7±7.5	14.2±7.0	12.5±7.4
Patients remission (18-19)	10.5±9.0 ^a	10.9±6.9*	13.6±7.6	12.6±6.5	12.2±4.9	8.9±3.7

Cortisol during the day

Daily secretion of cortisol was higher on Day 1 in patients with TRUD compared to those in remission ($t=1.970$, $d.f.=43$, $p=0.05$, 31 patients 98.0 ± 48.3 nmol/l.h vs 19 patients 78.9 ± 19.2 nmol/l.h). It was not different between groups for the rest of the days (Day 2, $t=1.946$, $d.f.=44$, $p=0.06$, 30 patients 95.2 ± 33.3 nmol/l.h vs 16 patients 76.6 ± 25.2 nmol/l.h; Day 3, $t=1.806$, $d.f.=38$, $p=0.08$, 25 patients 93.5 ± 27.4 nmol/l.h vs 15 patients 77.3 ± 27.7 nmol/l.h; Day 4, $t=-.274$, $d.f.=38$, $p=0.79$, 25 patients 88.5 ± 32.7 nmol/l.h vs 15 patients 85.8 ± 24.1 nmol/l.h) (Figure 7).

Single concentrations of cortisol in the morning, noon and evening are shown in Table 33.

Table 33. Cortisol concentrations (nmol/l) in patients with Treatment Resistant Unipolar Depression and remission (number of subjects, at morning, noon and evening over 4 days. All data are given as means±standard deviations, except for those indicated with a symbol ^a (medians±IQR), for which data a Mann-Whitney test is used.

<i>Cortisol</i>						
Day 1 (no of subjects)	awakening	p value	noon	p value	10pm	p value
Patients acute (33-34)	11.2±11.0 ^a		5.8±5.8 ^a		2.4±2.2 ^a	
Patients remission (19-21)	10.6±9.3 ^a	ns ^a	5.5±3.2 ^a	ns ^a	2.6±2.3 ^a	ns ^a
Day 2 (no of subjects)						
Patients acute (33-35)	10.9±7.7 ^a		6.9±3.6		2.2±1.9 ^a	
Patients remission (18-19)	10.5±9.0 ^a	ns ^a	5.1±3.0	ns	1.9±1.9 ^a	ns ^a
Day 3 (no of subjects)						
Patients acute (29-33)	10.4±7.1 ^a		5.4±3.9 ^a		2.9±2.0	
Patients remission (16-20)	9.5±8.6 ^a	ns ^a	5.2±2.1 ^a	ns ^a	2.4±1.2	ns
Day 4 (no of subjects)						
Patients acute (28-34)	12.7±7.0		5.9±2.7		2.3±3.1 ^a	
Patients remission (17-18)	11.8±4.5	ns	5.4±2.5	ns	2.5±1.6 ^a	ns ^a

DHEA

Single concentrations of DHEA on awakening and at 15, 30, 45, 60, 90 minutes

following awakening did not differ between patients and those in remission (Table 34).

Table 34. DHEA concentrations (nmol/l) in patients with Treatment Resistant Unipolar Depression and those in remission. All data are given as means±standard deviations, except for those indicated with a symbol ^a (medians±IQR).

DHEA						
Day 1 (no of subjects)	Awakening	+15 mins	+30 mins	+45 mins	+60 mins	+90 mins
Patients acute (29-32)	1.1±1.4 ^a	1.0±1.2 ^a	0.9±1.2 ^a	0.8±0.8 ^a	0.7±0.9 ^a	0.7±0.6 ^a
Patients remission (16-18)	0.8±2.9 ^a	0.9±2.3 ^a	0.7±1.2 ^a	0.6±1.2 ^a	0.6±1.5 ^a	0.4±0.4 ^a
Day 2 (no of subjects)						
Patients acute (32-34)	1.1±1.6 ^a	1.0±1.2 ^a	0.9±0.9 ^a	0.8±0.7 ^a	0.8±0.7 ^a	0.7±0.9 ^a
Patients remission (14-15)	1.2±4.1 ^a	1.1±1.3 ^a	0.7±1.3 ^a	0.6±1.1 ^a	0.6±1.0 ^a	0.7±0.9 ^a

DHEA secretion post awakening (AUCg)

DHEA secretion in the morning following awakening (DHEA response to awakening) did not differ between subjects on any days (Day 1, $z=-1.334$, $p=0.18$; Day 2 $z=-.231$, $p=0.82$; Day 3, $z=-.377$, $p=0.70$; Day 4, $z=-.841$, $p=0.40$).

DHEA secretion during the day (AUCg)

Daily secretion of DHEA did not differ between subjects with TRUD and those in remission (Day 1, $t=1.062$, d.f.=38, $p=0.29$; Day 2, $z=-.412$, $p=0.68$).

Cortisol/DHEA ratio

There were no differences between groups on any days (data not shown).

Cortisol/DHEA ratio (AUCg/DHEA secretion) post awakening

The ratio of Cortisol/DHEA, measured by dividing the CAR (AUCg) by the output of DHEA following awakening was not different between patients on Day 1 ($z=-.119$, $p=0.90$), or Day 2 ($z=-.20$, $p=0.84$).

Cortisol/DHEA ratio (AUCg/DHEA secretion) during the day

Similarly the ratio of Cortisol/DHEA during the day measured by dividing the total output of cortisol and that of DHEA during the day (AUCg) did not differ between subjects with TRUD and those in remission on Day 1 ($z=-.408$, $p=0.68$), Day 2 ($z=-.343$, $p=0.73$), Day 3 ($z=-.925$, $p=0.35$), or Day 4 ($z=-.247$, $p=0.80$).

Cortisol following awakening Day 1

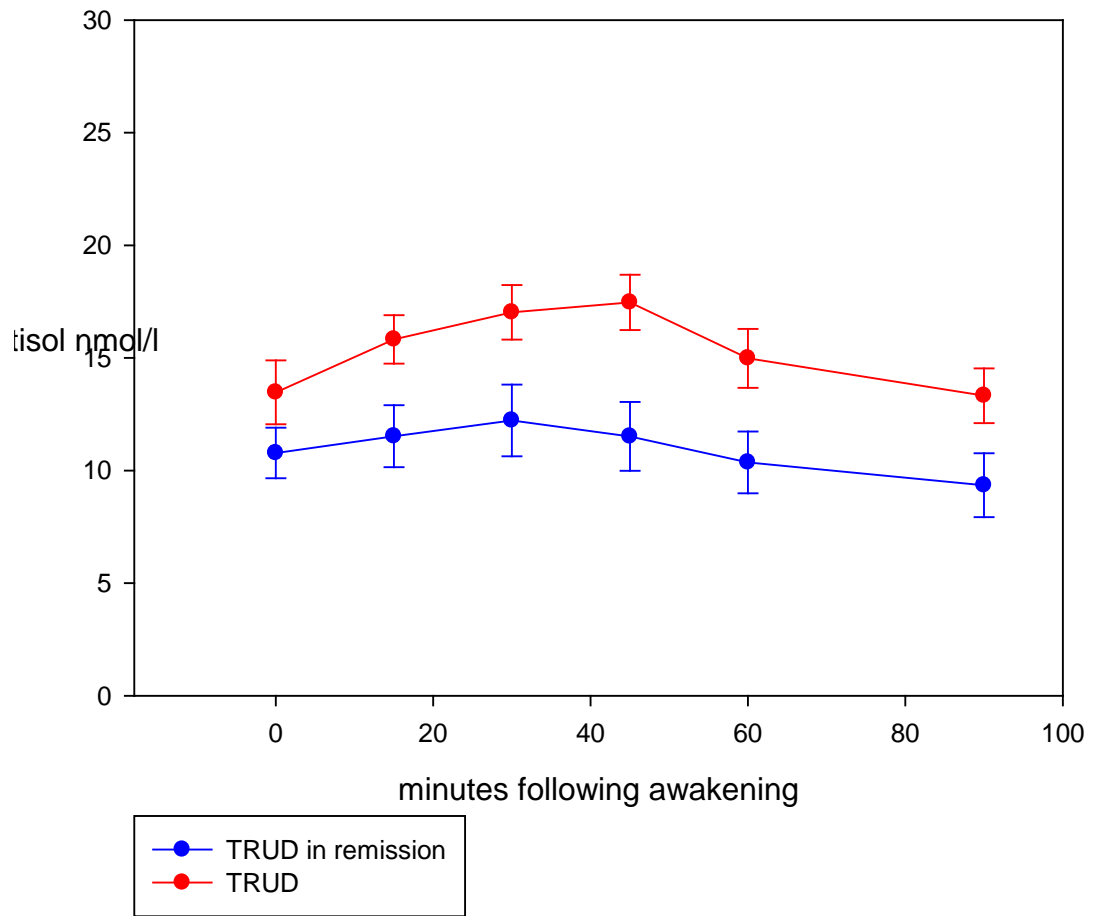


Figure 5. Cortisol concentrations (nmol/l) post awakening 0-90 minutes on Day 1 in 31 patients with Treatment Resistant Unipolar Depression and 19 patients in remission.

Cortisol following awakening Day 2

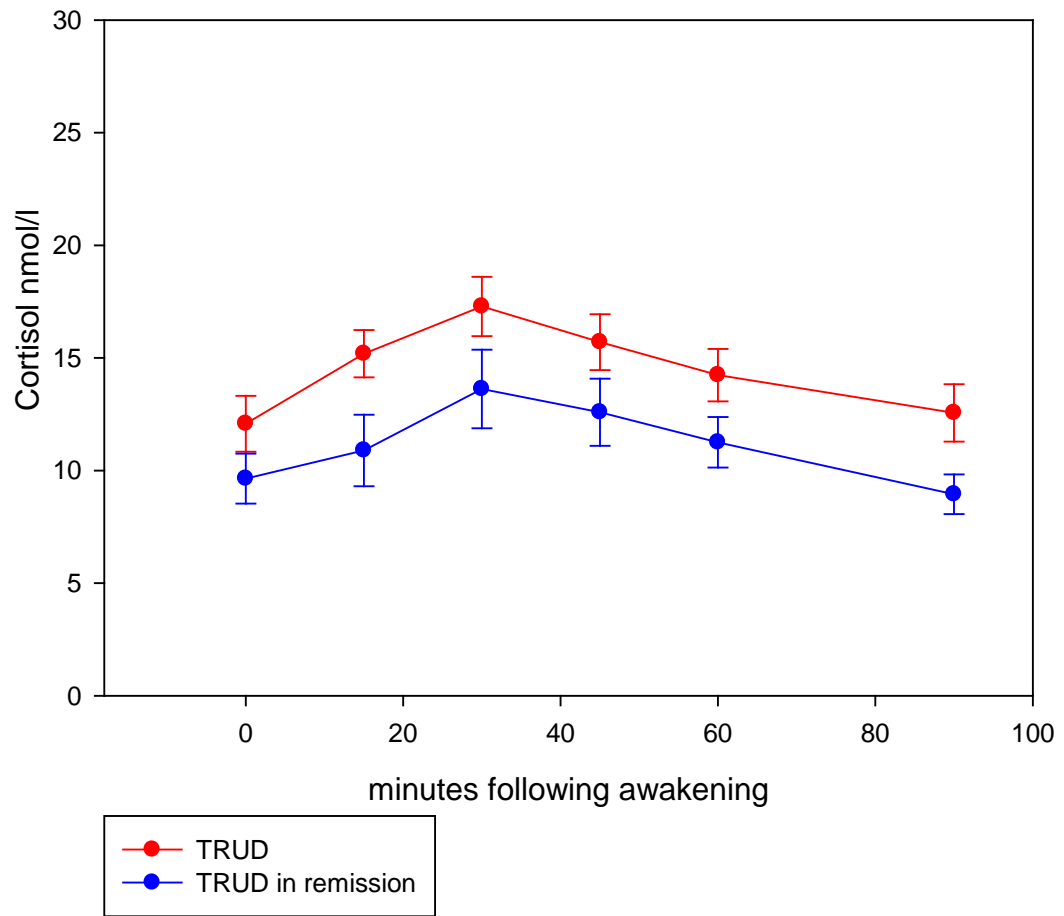


Figure 6. Cortisol concentrations (nmol/l) post awakening 0-90 minutes on Day 2 in 32 patients with Treatment Resistant Unipolar Depression and 18 patients in remission.

Cortisol during day Day 1

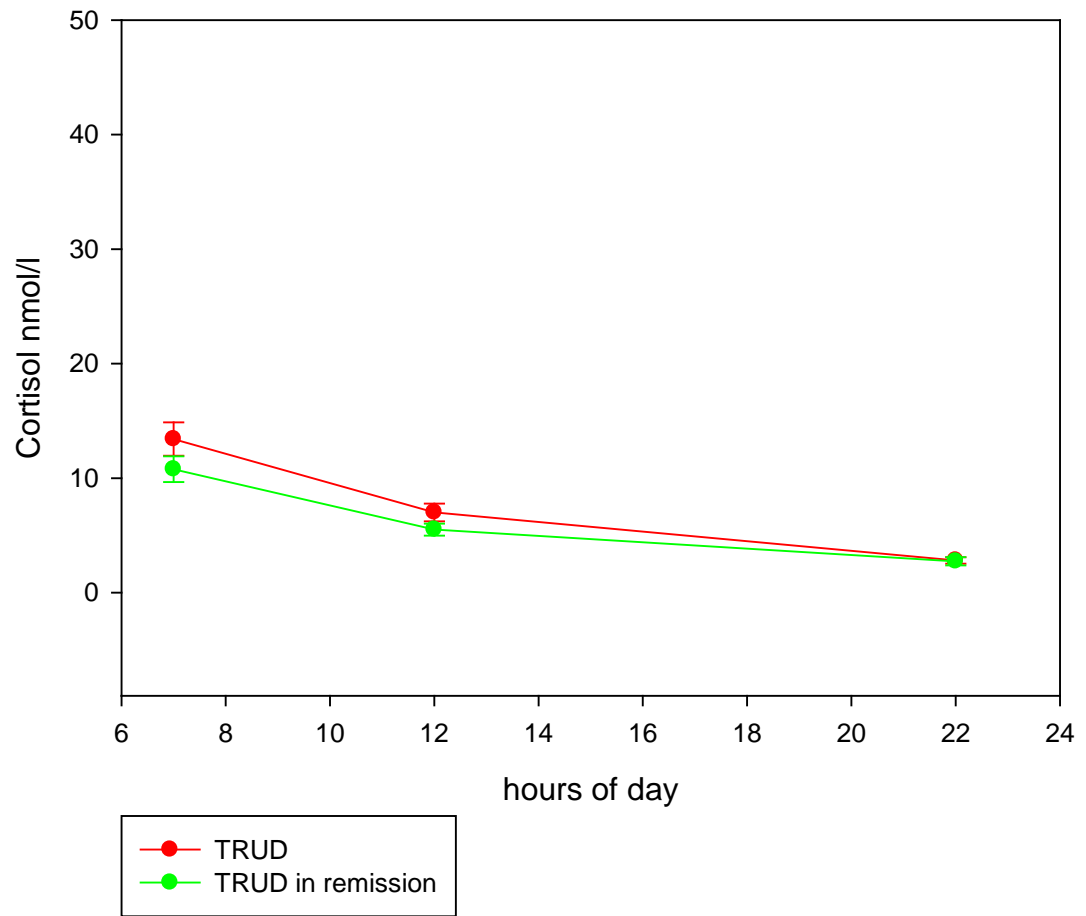


Figure 7. Cortisol concentrations (nmol/l) during the day in 31 patients with Treatment Resistant Unipolar Depression and 19 patients with Treatment Resistant Bipolar Depression on Day 1.

Comparison of patients with Treatment Resistant Bipolar Depression and those in remission

CAR

AUC_g

The CAR was lower in patients with TRBD compared to those in remission on Day 1 (Day 1, $z=-3.009$, $p=0.002$, 14 patients median (IQR) 915.4 (537.0) nmol/l.min vs 20 patients median (IQR) 1197.4 (505.5) nmol/l.min; Day 2, $t=-.381$, d.f.=33, $p=0.70$, 17 patients 1101.5±527.4 nmol/l.min vs 18 patients 1164.8±454.8 nmol/l.min; mean of 2 days $t=3.216$, d.f.=39, $p=0.004$, 14 patients 902.2±304.3 nmol/l.min vs 18 patients 1224.9±315.5 nmol/l.min).

AUC_i

The AUC_i was lower in subjects with TRBD compared those in remission on Day 1 ($z=-2.624$, $p=0.00$, 14 patients median (IQR) -209.2 (434.4) nmol/l.min vs 20 patients median (IQR) 247.5 (365.8) nmol/l.min), but it was not different on Day 2 ($t=.028$, df=33, $p=0.98$, 17 patients 53.2±628.0 nmol/l.min vs 18 patients 47.8±544.7 nmol/l.min), or once the mean of the 2 days was calculated ($t=-1.353$, d.f.=30, $p=0.20$, 14 patients -88.8±387.2 nmol/l.min vs 18 patients 110.4±432.2 nmol/l.min).

Salivary cortisol concentrations at 0, 15, 30, 45, 60 and 90 minutes post awakening

The concentration of cortisol post awakening was not different between subjects on Day 1 ($t=-1.055$, d.f.=33, $p=0.30$), or Day 2 ($t=-.348$, d.f.=33, $p=0.73$). Similarly there were no differences in cortisol between subjects on either days at 15 minutes following awakening (Day 1, $t=-2.064$, d.f.=33, $p=0.05$; or Day 2, $t=-.261$, d.f.=33, $p=0.80$), or at

30 minutes following awakening (Day 1, $z=-1.700$, $p=0.09$; Day 2, $t=-.164$, $d.f.=33$, $p=0.87$). Cortisol was higher in subjects with TRBD in remission compared to those in the acute phase of illness at 45 minutes following awakening on Day 1 ($t=-3.337$, $d.f.=32$, $p<0.001$), but not on Day 2 ($t=-.359$, $d.f.=33$, $p=0.72$). Similarly it was higher at 60 minutes following awakening in subjects during the remitted phase of their illness on Day 1 ($z=-3.641$, $p<0.001$), but not on Day 2 ($t=-.385$, $d.f.=33$, $p=0.70$) and also at 90 minutes following awakening on Day 1 ($t=-3.376$, $d.f.=32$, $p<0.001$), but not on Day 2 ($z=-1.238$, $p=0.21$).

Single cortisol values following awakening are shown in Table 35 and Figures 8 and 9.

Table 35. Cortisol concentrations (nmol/l) in patients with Treatment Resistant Bipolar Depression and those in remission. All data are given as means \pm standard deviations, except for those indicated with a symbol ^a (medians \pm IQR).

<i>Cortisol</i>						
Day 1 (no of subjects)	Awakening	+15 mins	+30 mins	+45 mins	+60 mins	+90 mins
Patients acute (14-15)	10.5 \pm 4.8	12.9 \pm 5.3	12.5 \pm 7.0 ^a	9.7 \pm 4.5	8.6 \pm 2.9 ^a	6.2 \pm 2.6
Patients remission (20)	12.3 \pm 5.2	16.8 \pm 5.9	15.5 \pm 6.7 ^a	15.2 \pm 4.9	12.7 \pm 4.4 ^a	10.9 \pm 4.7
Day 2 (no of subjects)						
Patients acute (17)	11.6 \pm 5.9	13.6 \pm 6.0	14.5 \pm 7.9	12.4 \pm 8.1	12.0 \pm 6.9	7.8 \pm 5.7 ^a
Patients remission (18)	12.4 \pm 7.0	14.2 \pm 7.5	14.9 \pm 7.4	13.2 \pm 5.5	12.0 \pm 6.1	8.9 \pm 3.5 ^a

Cortisol during the day (AUCg)

Secretion of cortisol (AUCg) was higher on Day 1 in patients with TRBD in remission ($t=-2.489$, d.f.=25, $p=0.02$, 11 patients 75.3 ± 17.6 nmol/l.h vs 16 patients 98.6 ± 27.6 nmol/l.h). It was not different between groups on the rest of the days measured (Day 2, $t=-1.896$, d.f.=26, $p=0.07$, 16 patients 78.8 ± 19.3 nmol/l.h vs 16 patients 95.6 ± 29.6 nmol/l.h; Day 3, $t=1.129$, d.f.=17, $p=0.27$, 8 patients 90.3 ± 20.1 nmol/l.h vs 11 patients 79.0 ± 22.4 nmol/l.h; Day 4, $t=.064$, d.f.=19, $p=0.95$, 8 patients 90.5 ± 44.9 nmol/l.h vs 13 patients 89.5 ± 31.3 nmol/l.h). The small numbers on Day 3 and 4 make these results difficult to interpret.

Single values of cortisol in the morning, noon and evening are described at Table 36 and shown in Figures 10 and 11.

Table 36. Cortisol concentrations (nmol/l) in patients with Treatment Resistant Bipolar Depression and remission in the morning, noon and evening over 4 days. All data are given as means±standard deviations, except for those indicated with a symbol ^a (medians±IQR), for which data a Mann-Whitney test is used.

<i>Cortisol</i>						
Day 1 (no of subjects)	awakening	p value	noon	p value	10pm	p value
Patients acute (13-15)	10.5±4.8		5.3±2.9		2.7±1.9	
Patients remission (17-20)	12.3±5.2	ns	7.4±3.1	0.07	2.7±1.9	ns
Day 2 (no of subjects)						
Patients acute (16-17)	11.6±5.9		5.3±2.0		2.5±1.5	
Patients remission (16-18)	12.4±7.0	ns	6.8±2.6	0.05	2.8±1.7	ns
Day 3 (no of subjects)						
Patients acute (12-14)	12.6±7.4		5.8±2.1		3.2±1.7	
Patients remission (15-16)	13.5±6.9	ns	6.1±2.5	ns	1.8±0.9	0.03
Day 4 (no of subjects)						
Patients acute (12-13)	12.5±5.3		5.7±3.5		2.7±2.2	
Patients remission (15-17)	14.6±6.9	ns	6.3±3.1	ns	2.1±1.0	ns

Daily secretion of DHEA

Values of DHEA are shown in Table 36 and 38.

Single concentrations of DHEA on awakening and at 15, 30, 45, 60, 90 minutes following awakening did not differ between subjects (Table 37).

Table 37. DHEA concentrations (nmol/l) in patients with Treatment Resistant Bipolar Depression and those in remission. All data are given as means±standard deviations, except for those indicated with a symbol ^a (medians±IQR).

<i>DHEA</i>						
Day 1 (no of subjects)	Awakening	+15 mins	+30 mins	+45 mins	+60 mins	+90 mins
Patients acute (14)	1.4±1.1	1.2±1.0	0.6±1.3 ^a	0.7±0.6	0.5±1.0 ^a	0.6±0.4
Patients remission (16-17)	1.4±1.0	1.4±1.0	1.0±1.4 ^a	1.2±1.1	0.6±1.2 ^a	0.8±0.6
Day 2 (no of subjects)						
Patients acute (14-16)	1.5±2.2 ^a	1.0±1.7 ^a	0.8±1.3 ^a	0.7±0.6 ^a	0.9±0.8 ^a	0.8±0.8 ^a
Patients Remission (15-17)	0.8±0.8 ^a	0.9±1.4 ^a	0.7±1.9 ^a	0.5±0.7 ^a	0.7±1.4 ^a	0.6±1.0 ^a

Table 38. DHEA concentrations (nmol/l) in patients with Treatment Resistant Bipolar Depression and remission (number of subjects, at morning, noon and evening over 4 days. All data are given as medians±IQR, for which data a Mann-Whitney test is used.

DHEA						
Day 1 (no of subjects)	awakening	p value	noon	p value	10pm	p value
Acute (29-32)	1.1±1.4 ^a		0.6±0.7 ^a		0.4±0.5 ^a	
Remission (16-18)	1.0±1.0 ^a	ns ^a	0.5±0.4 ^a	ns ^a	1.1±1.6 ^a	ns ^a
Day 2 (no of subjects)						
Acute (31-33)	1.1±1.6 ^a		0.6±0.6 ^a		0.4±0.5 ^a	
Remission (14-15)	1.2±4.1 ^a	ns ^a	0.3±0.5 ^a	ns ^a	0.3±0.4 ^a	ns ^a
Day 3 (no of subjects)						
Acute (25-29)	1.4±1.5 ^a		0.6±0.5 ^a		0.3±0.2 ^a	
Remission (13-17)	1.2±3.1 ^a	ns ^a	0.9±1.1 ^a	ns ^a	0.3±0.6 ^a	ns ^a
Day 4 (no of subjects)						
Acute (21-26)	1.2±1.1 ^a		0.6±0.8 ^a		0.3±0.4 ^a	
Remission (15)	1.2±1.4 ^a	ns ^a	0.7±1.1 ^a	ns ^a	0.4±0.7 ^a	ns ^a

DHEA secretion post awakening (AUCg)

DHEA secretion in the morning following awakening (DHEA response to awakening) did not differ between subjects with Bipolar Depression (Day 1, $t=-.665$, d.f.=28, $p=0.51$; Day 2, $z=-1.149$, $p=0.25$).

DHEA secretion during the day (AUCg)

Daily secretion of DHEA did not differ between subjects with Bipolar Depression (Day 1, $t=.362$, $d.f.=23$, $p=0.72$; Day 2, $t=1.696$, $d.f.=25$, $p=0.10$; Day 3, $z=-.866$, $p=0.39$; Day 4, $z=-1.00$, $p=0.31$).

Cortisol/DHEA ratio

Daily values of Cortisol/DHEA are shown in Table 39. The cortisol/DHEA ratio was higher in patients with TRBD compared to remitted patients on Days 2 and 4 and lower in remission at noon on Day 4.

Table 39. Cortisol/DHEA ratio in patients with Treatment Resistant Bipolar Depression and remission (number of subjects, at morning, noon and evening over 4 days. All data are given as medians±IQR, for which data a Mann-Whitney test is used.

<i>Cortisol/DHEA</i>						
Day 1 (no of subjects)	awakening	p value	noon	p value	10pm	p value
Acute (28-31)	8.9±12.6 ^a		9.3±13.5 ^a		5.0±10.4 ^a	
Remission (15-18)	13.0±20.1 ^a	ns ^a	10.9±17.5 ^a	ns ^a	6.3±10.9 ^a	ns ^a
Day 2 (no of subjects)						
Acute (29-33)	8.2±10.5 ^a		12.2±19.4 ^a		6.5±7.3 ^a	
Remission (14-15)	6.5±15.3 ^a	0.05 ^a	16.2±16.6 ^a	0.03 ^a	6.5±11.6 ^a	ns ^a
Day 3 (no of subjects)						
Acute (11-13)	11.1±9.7 ^a		8.0±13.5 ^a		5.5±8.1 ^a	
Remission (13-17)	5.5±12.5 ^a	0.01 ^a	5.2±9.4 ^a	ns ^a	5.7±7.2 ^a	ns ^a
Day 4 (no of subjects)						
Acute (12-15)	11.0±10.3 ^a		6.5±4.0 ^a		7.3±5.6 ^a	
Remission (14-15)	10.1±7.3 ^a	ns ^a	13.8±14.0 ^a	ns ^a	5.4±9.5 ^a	ns ^a

Cortisol/DHEA ratio (AUCg/DHEA secretion) post awakening

The ratio of Cortisol/DHEA measured by dividing the CAR (AUCg) in the morning with the output of DHEA following awakening was not different between subjects on Day 1 ($z=-.877$, $p=0.38$), or on Day 2 ($z=-1.700$, $p=0.09$), or when the mean of the 2 days was calculated ($z=-2.026$, $p=0.04$).

Cortisol/DHEA ratio (AUCg/DHEA secretion) during the day

Similarly the ratio of Cortisol/DHEA during the day measured by dividing the total output of cortisol and that of DHEA during the day (AUCg) did not differ between subjects on Day 1 ($z=-1.674$, $p=0.09$), Day 2 ($z=-1.800$, $p=0.07$), Day 3 ($z=-1.429$, $p=0.15$), Day 4 ($z=-1.389$, $p=0.16$).

Cortisol following awakening Day 1

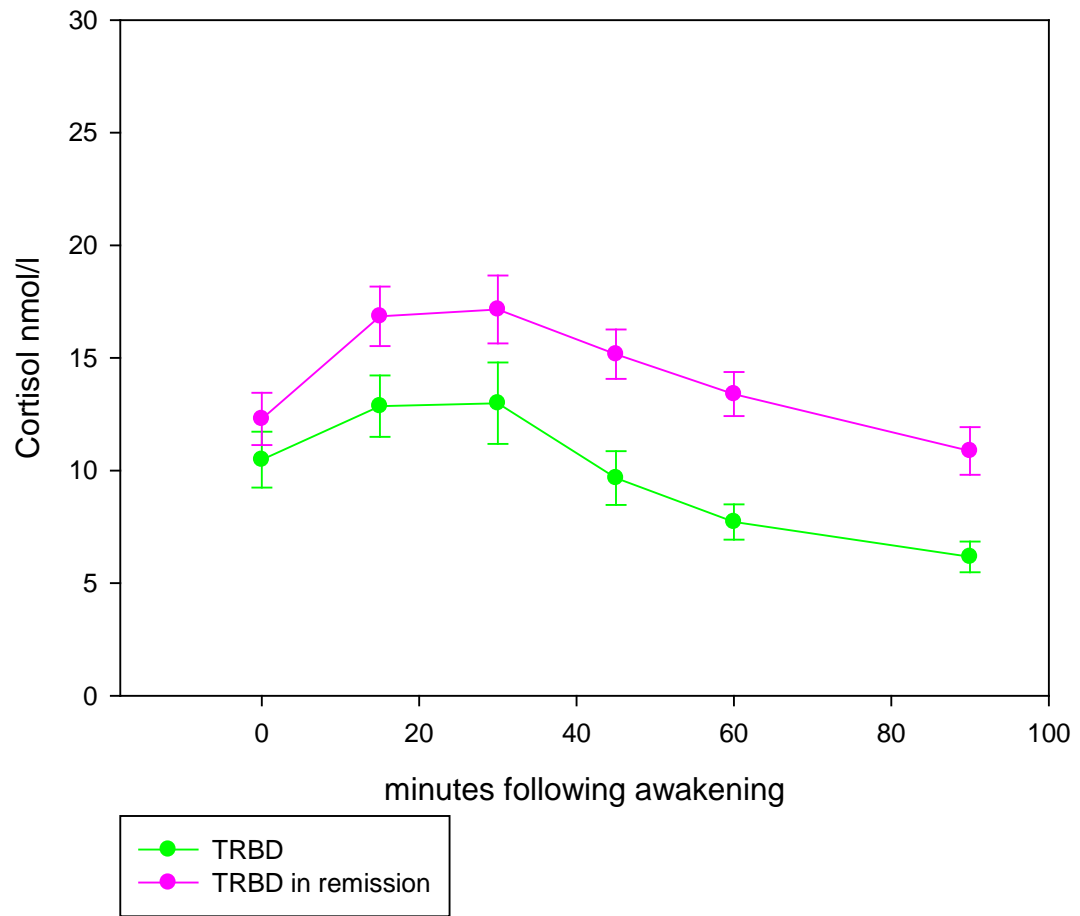


Figure 8. Cortisol concentrations (nmol/l) post awakening 0-90 minutes on Day 1 in 14 patients with Treatment Resistant Bipolar Depression and 20 patients in remission.

Cortisol during day Day 2

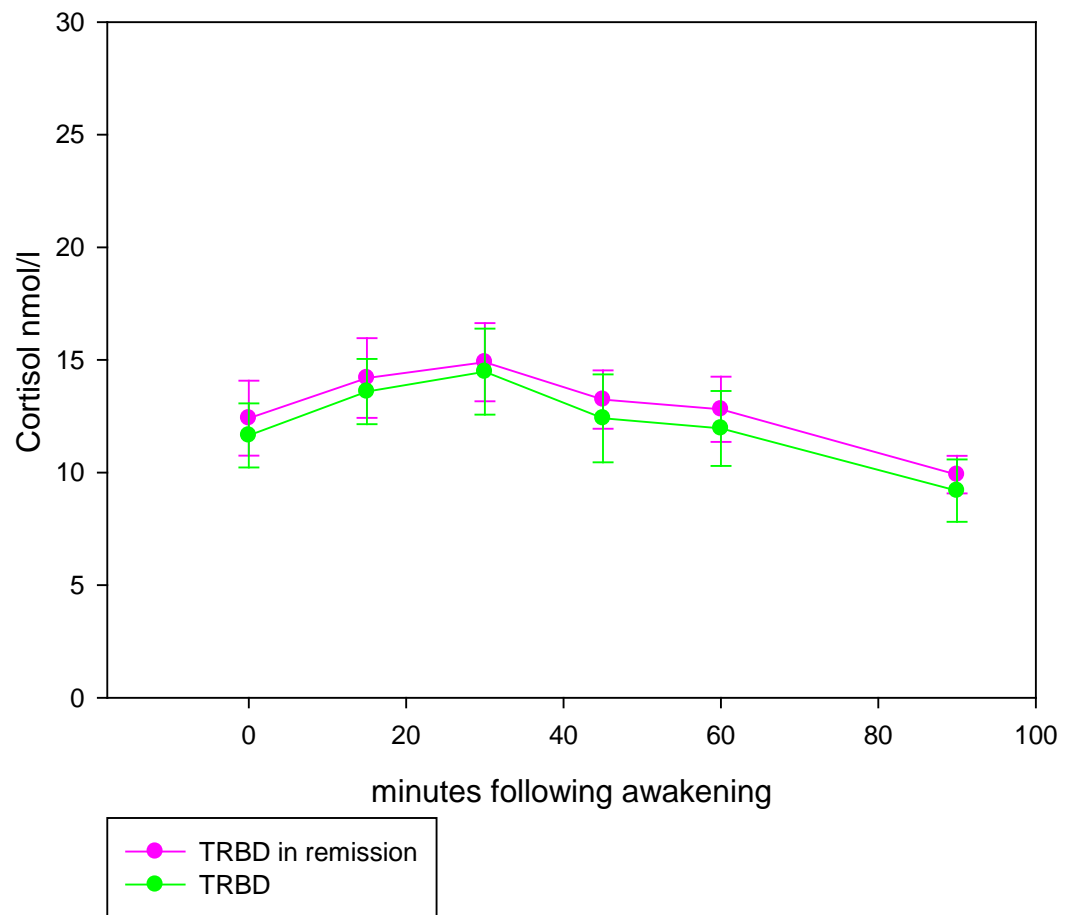


Figure 9. Cortisol concentrations (nmol/l) post awakening 0-90 minutes on Day 2 in 14 patients with Treatment Resistant Bipolar Depression and 20 patients in remission.

Cortisol during day Day 1

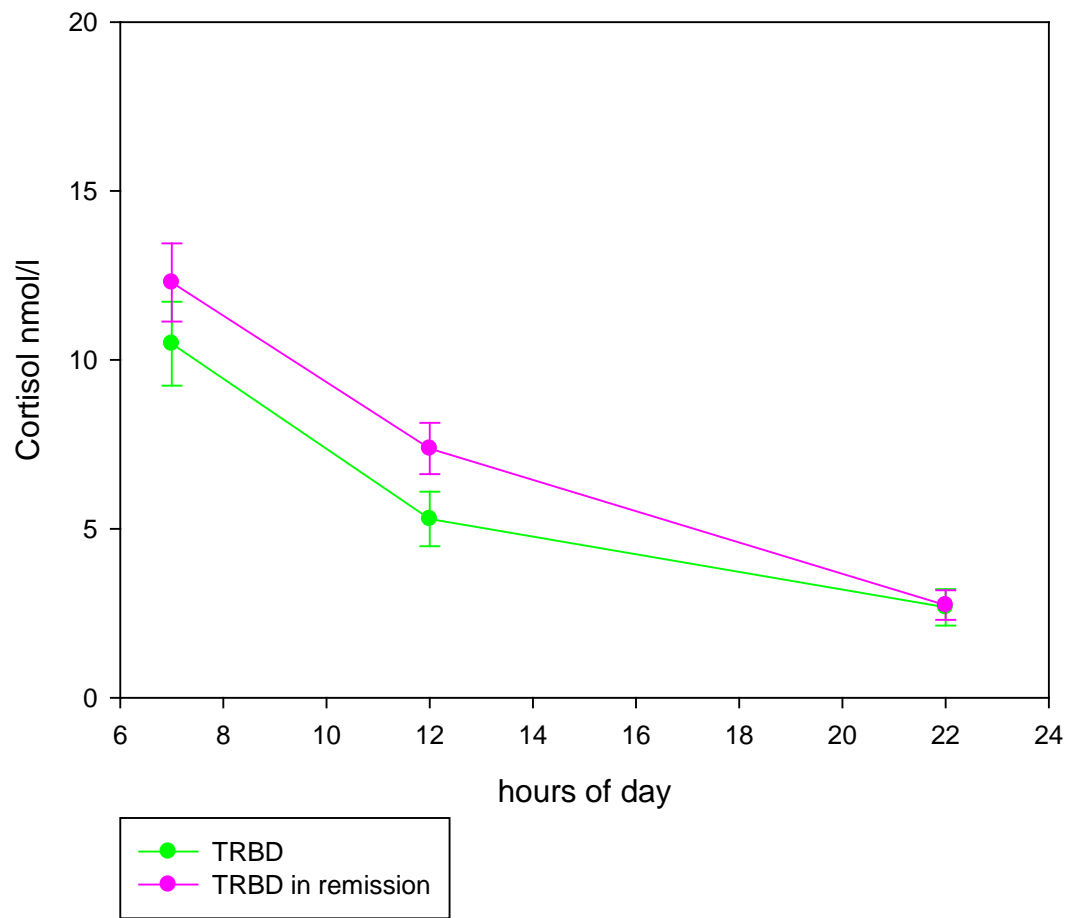


Figure 10. Cortisol concentrations (nmol/l) during the day on Day 1 in 11 patients with Treatment Resistant Bipolar Depression and 16 patients in remission.

Cortisol during day Day 2

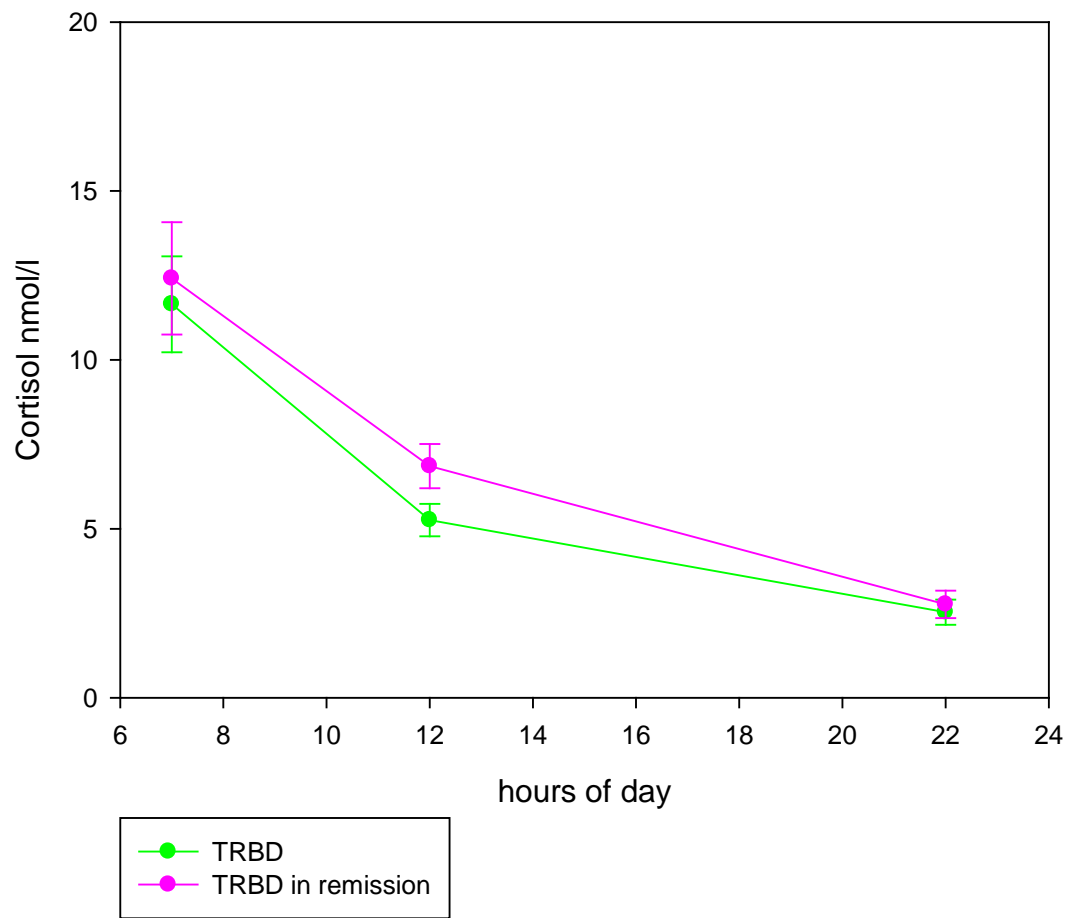


Figure 11. Cortisol concentrations (nmol/l) during the day on Day 2 in 16 patients with Treatment Resistant Bipolar Depression and 16 patients in remission.

Cortisol following awakening Day 1

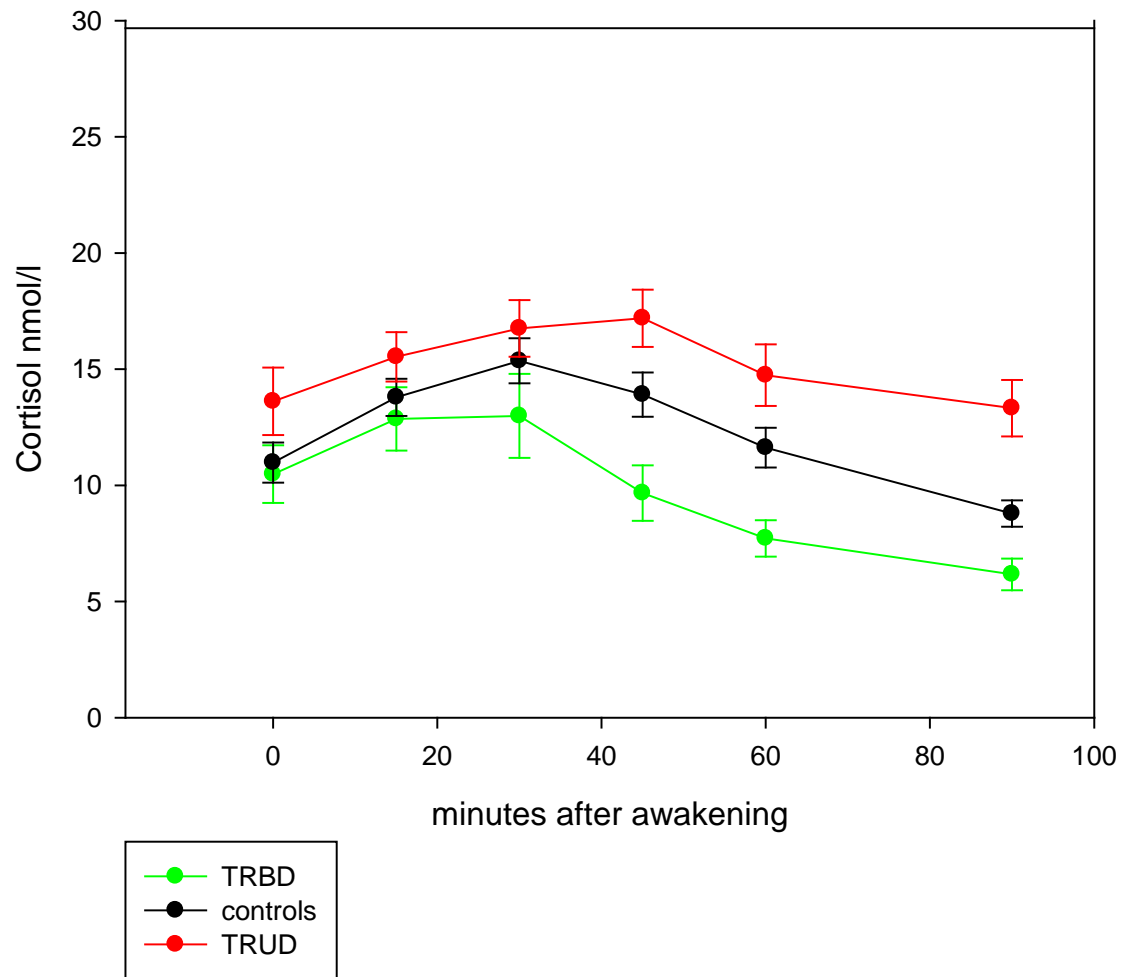


Figure 12. Cortisol concentrations (nmol/l) post awakening 0-90 minutes on Day 1 in 31 patients with Treatment Resistant Unipolar Depression, 44 controls and 14 patients with Treatment Resistant Bipolar Depression.

Cortisol following awakening Day 2

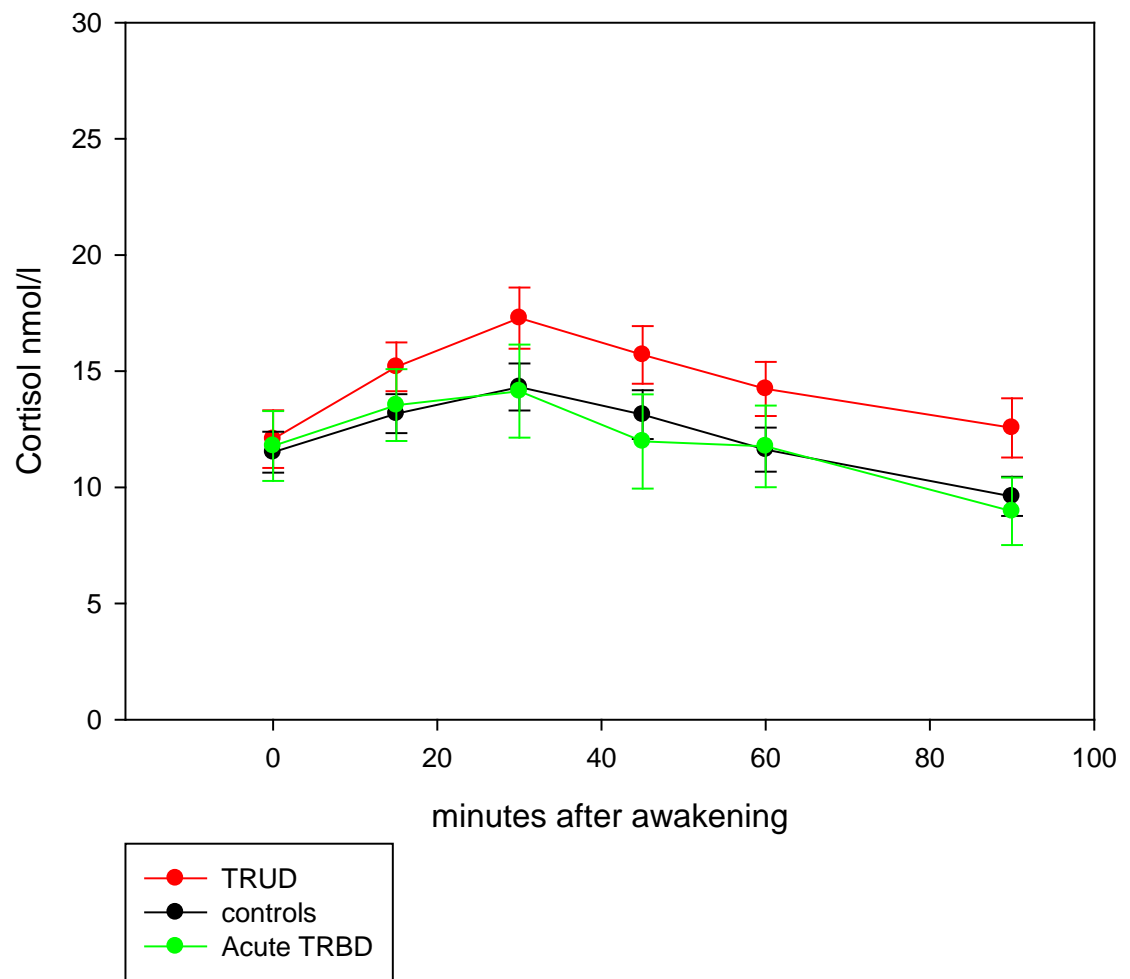


Figure 13. Cortisol concentrations (nmol/l) post awakening 0-90 minutes on Day 2 in 32 patients with Treatment Resistant Unipolar Depression, 40 controls and 17 patients with Treatment Resistant Bipolar Depression.

Cortisol during day Day 1

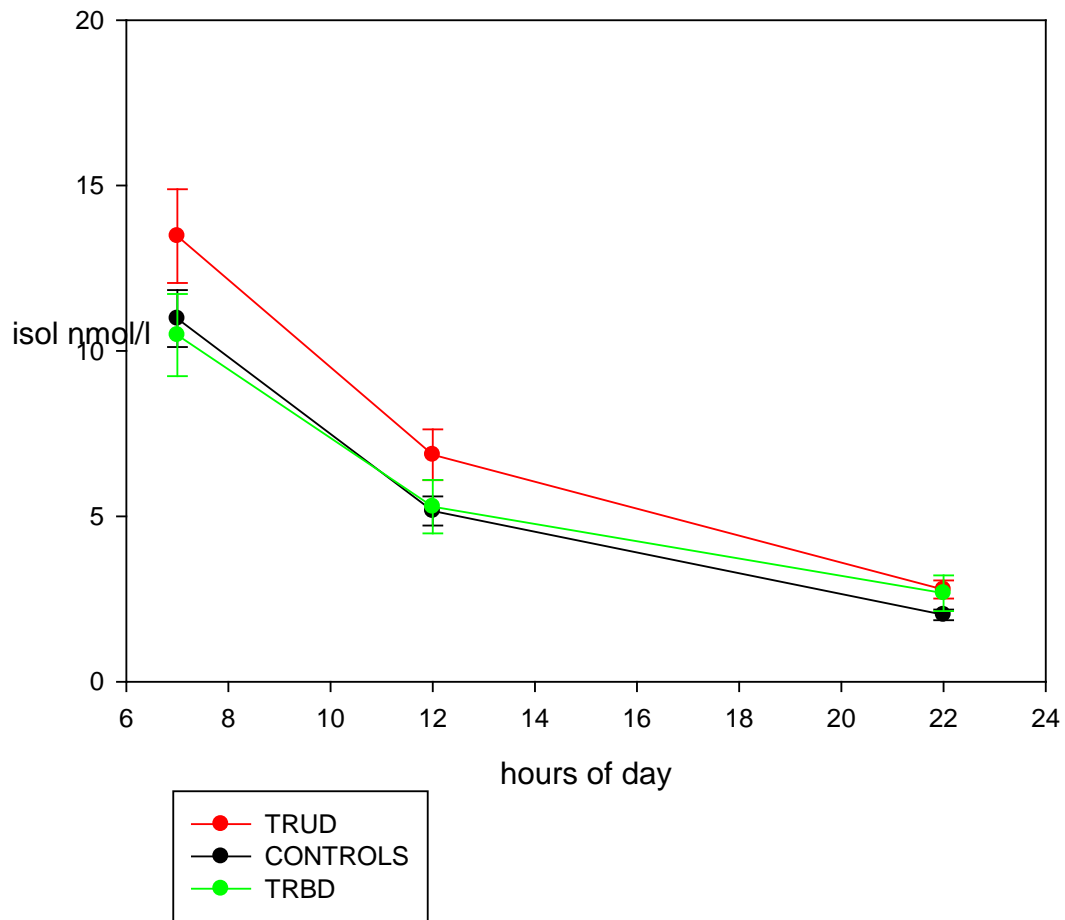


Figure 14. Cortisol concentrations (nmol/l) post awakening 0-90 minutes on Day 1 in 31 patients with Treatment Resistant Unipolar Depression, 40 controls and 11 patients with Treatment Resistant Bipolar Depression.

Cortisol during day Day 2

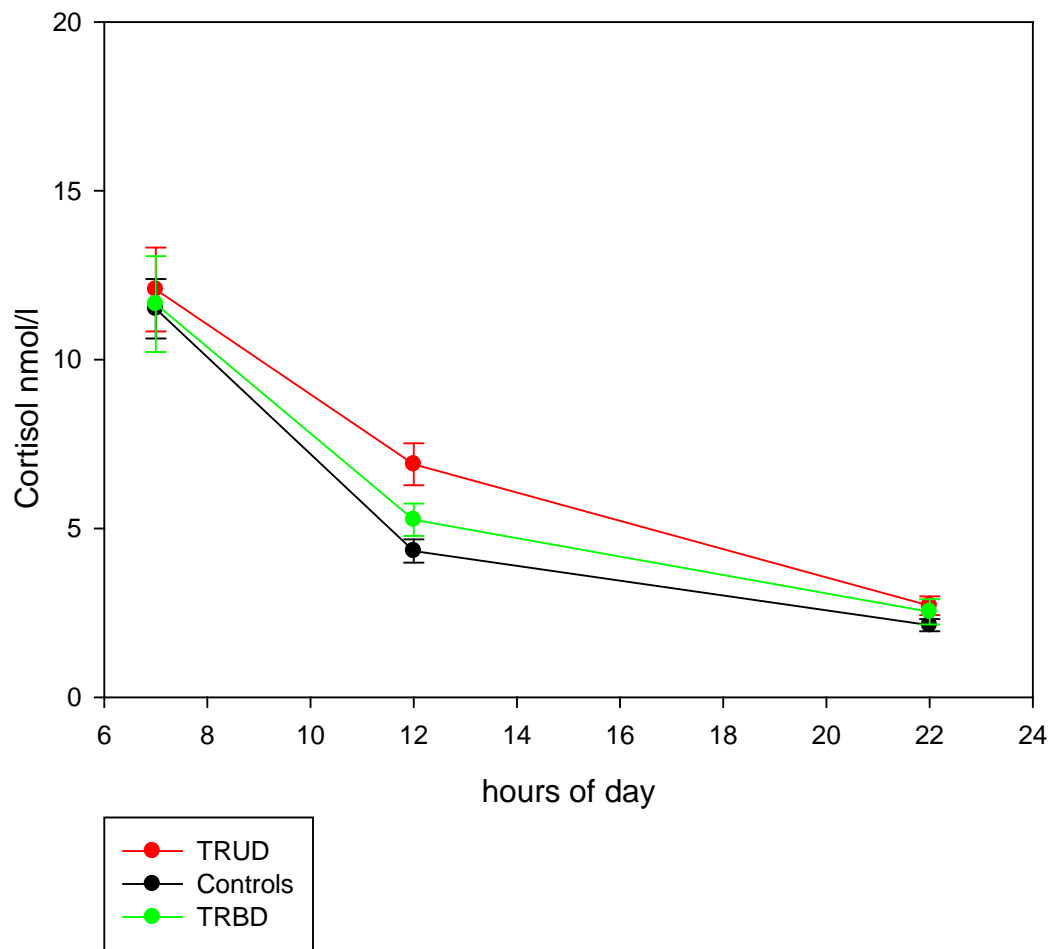


Figure 15. Cortisol concentrations (nmol/l) post awakening 0-90 minutes on Day 2 in 30 patients with Treatment Resistant Unipolar Depression, 40 controls and 16 patients with Treatment Resistant Bipolar Depression.

Cortisol following awakening Day 1

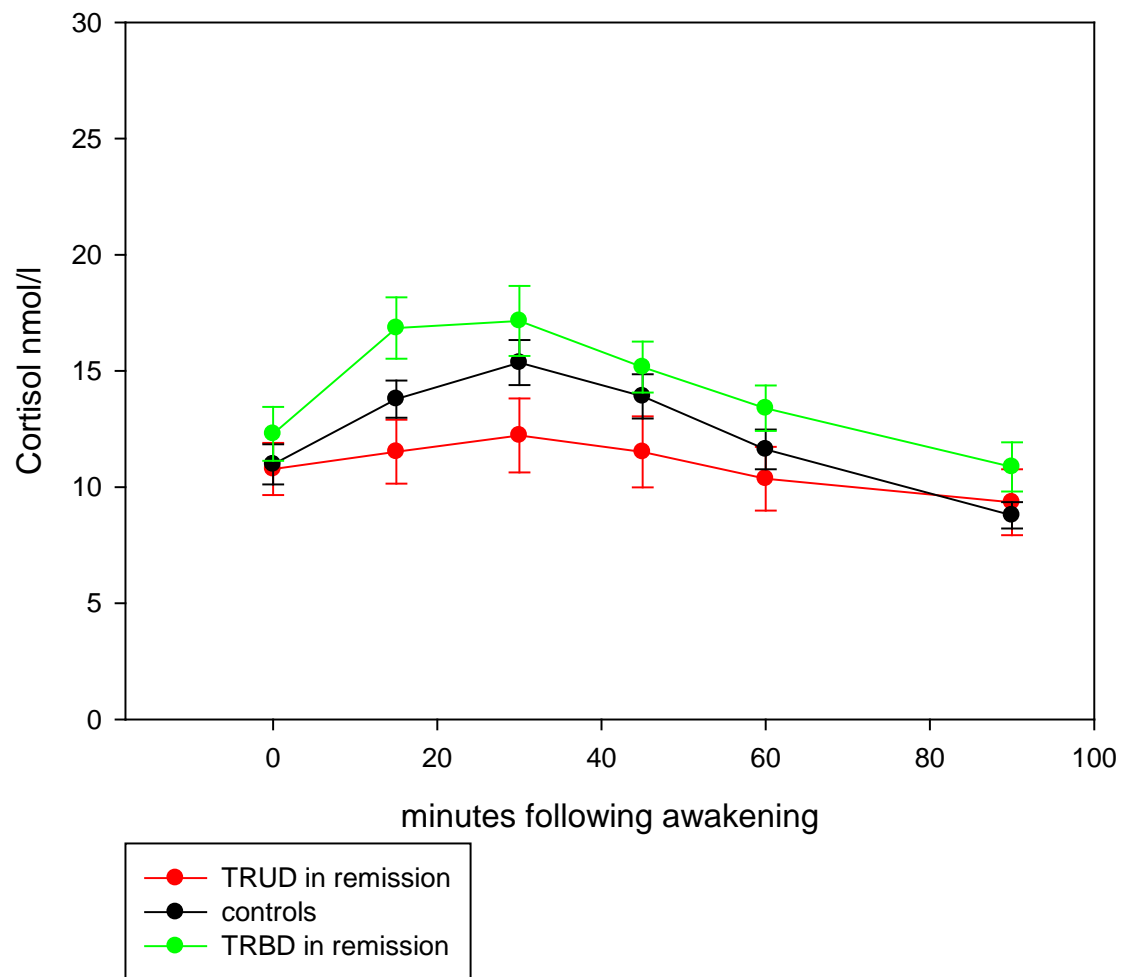


Figure 16. Cortisol concentrations (nmol/l) post awakening 0-90 minutes on Day 1 in 31 patients with Treatment Resistant Unipolar Depression and 14 patients with Treatment Resistant Bipolar Depression and 44 controls.

Cortisol following awakening Day 2

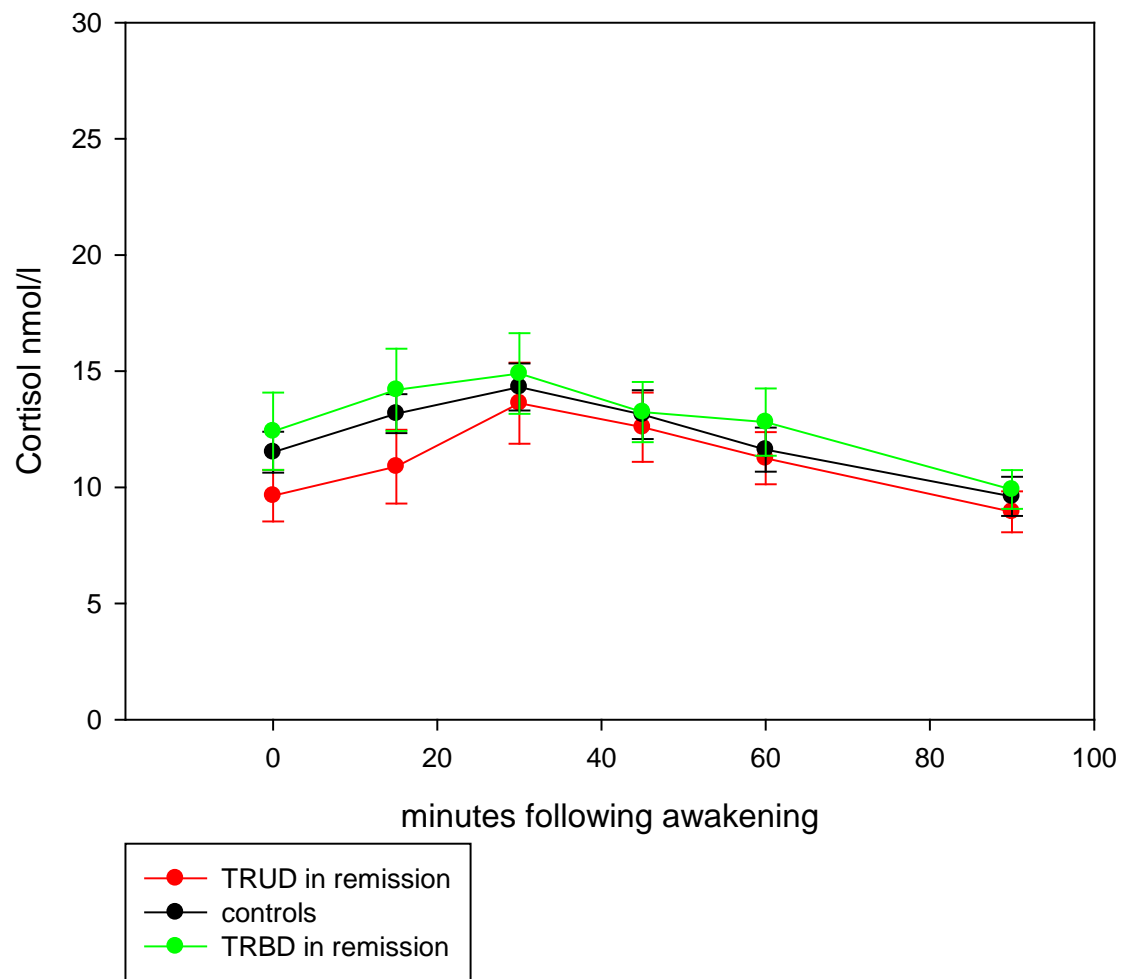


Figure 17. Cortisol concentrations (nmol/l) post awakening 0-90 minutes on Day 2 in 30 patients with Treatment Resistant Unipolar Depression and 17 patients with Treatment Resistant Bipolar Depression and 40 controls.

Cortisol during day Day 1

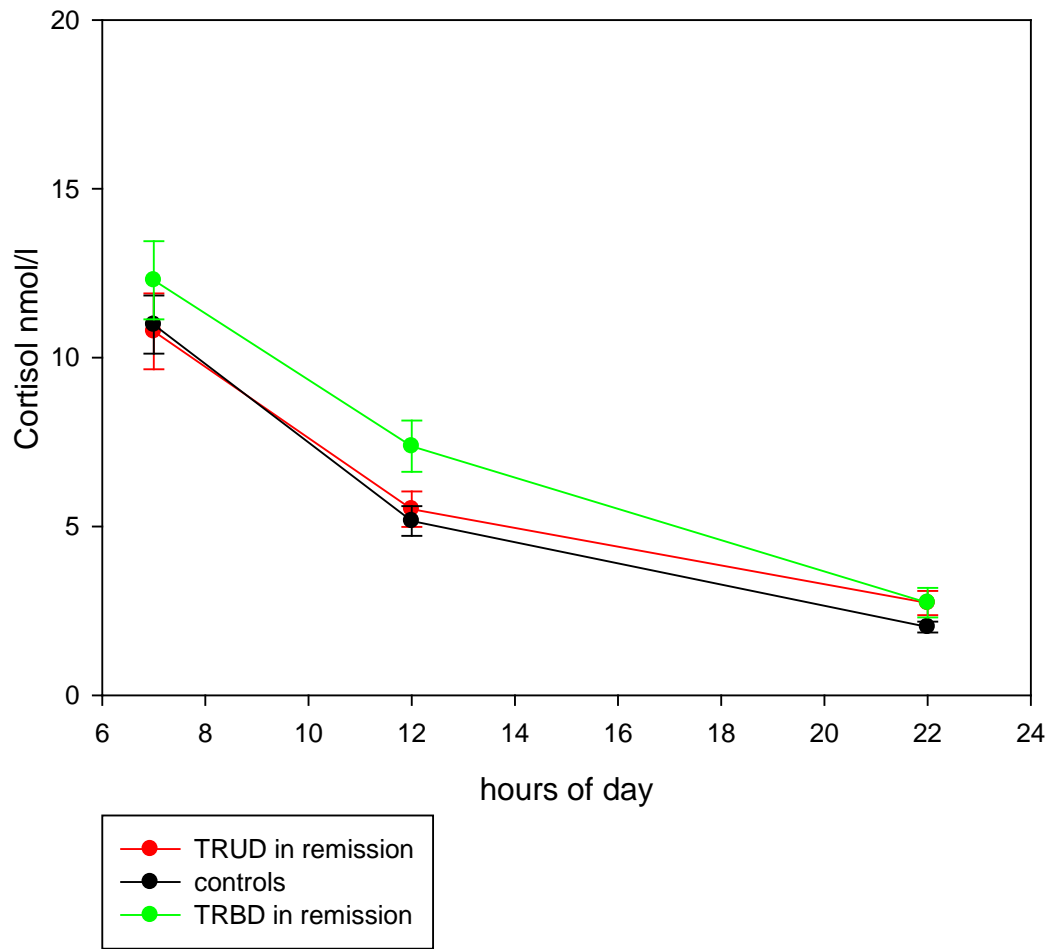


Figure 18. Cortisol concentrations (nmol/l) post awakening 0-90 minutes on Day 1 in 19 patients with Treatment Resistant Unipolar Depression, 43 controls and 16 patients with Treatment Resistant Bipolar Depression.

Cortisol during day Day 2

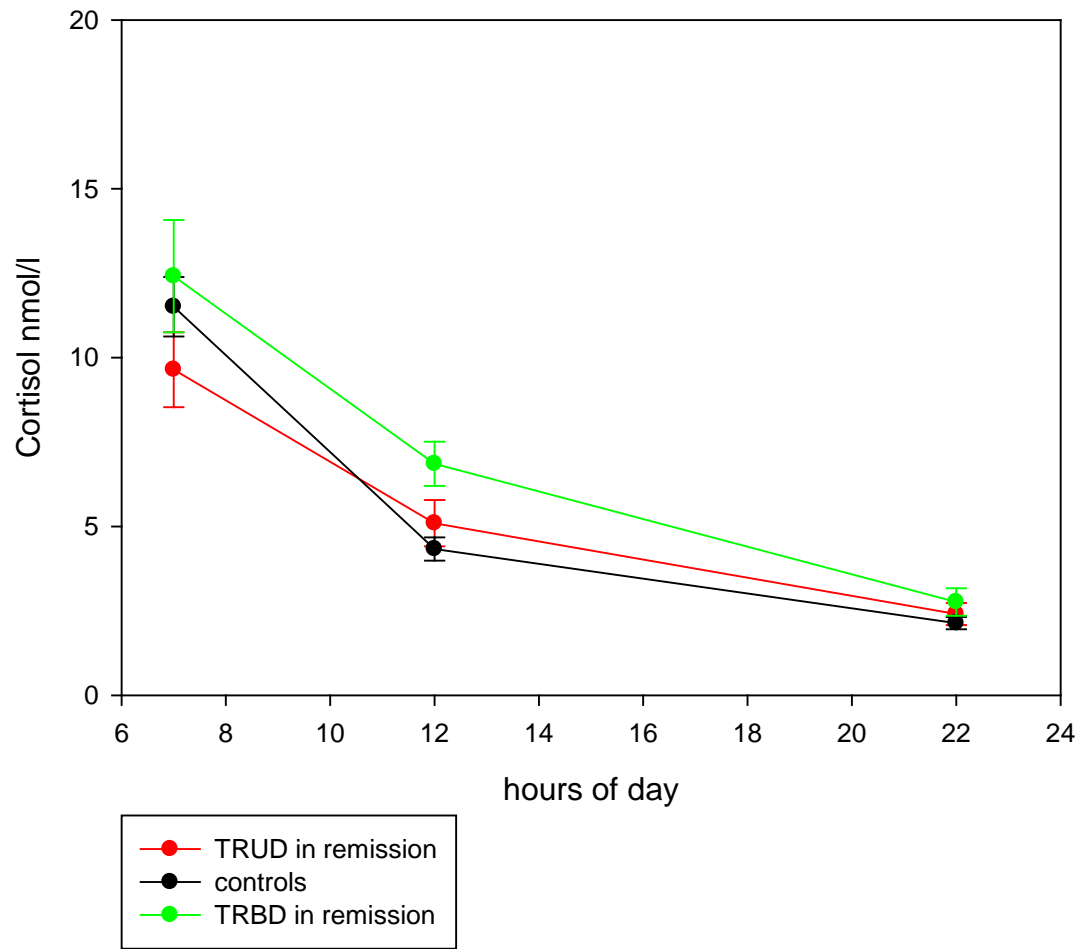


Figure 19. Cortisol concentrations (nmol/l) post awakening 0-90 minutes on Day 1 in 16 patients with Treatment Resistant Unipolar Depression, 40 controls and 16 patients with Treatment Resistant Bipolar Depression.

Other variables in subjects with Treatment Resistant Unipolar Depression

As mentioned in the Methodology Chapter, a correction for multiple comparisons using the rough false discovery rate i.e. the α - value being adjusted by $(n+1)/2n$, which for 84 tests gave an adjusted significance level of $p < 0.025$ was used and correlations below that value were deemed to be significant. Only the AUCg post awakening and daily secretion is described below.

Smoking

Smoking (smoking more than 10 cigarettes/day) did not have any significant effect on cortisol (7 smokers and 20 non smokers) (AUCg post awakening, Day 1, $z = -.522$, $p = 0.60$; Day 2, $z = -.118$, $p = 0.90$; AUCg for daily cortisol secretion on Day 1, $z = -.851$, $p = 0.39$ and on Day 2, $z = -.140$, $p = 0.89$), DHEA (DHEA secretion on Day 1 following awakening, $z = -.648$, $p = 0.52$ and on Day 2, $z = -.378$, $p = 0.70$; AUCg for daily DHEA secretion on Day 1, $z = -1.200$, $p = 0.23$ and on Day 2, $z = -.274$, $p = 0.78$), or the ratio of Cortisol/DHEA.

Place of collection

There were no hormonal differences between subjects who collected on the ward or at home (24 patients collected at home and 13 on the ward) on cortisol levels (AUCg post awakening, Day 1, $z = -.391$, $p = 0.69$, Day 2, $z = -.170$, $p = 0.86$; AUCg for daily cortisol secretion on Day 1, $z = -1.379$, $p = 0.16$ and on Day 2, $z = -.481$, $p = 0.63$). Similarly there were no differences on DHEA levels (DHEA secretion on Day 1 following awakening $z = -.165$, $p = 0.87$ and on Day 2, $z = -.00$, $p = 1.00$; AUCg for daily DHEA secretion on Day 1, $z = -.954$, $p = 0.34$ and on Day 2, $z = -.085$, $p = 0.93$), or the ratio of Cortisol/DHEA.

Day of collection

An analysis was conducted to determine whether there were hormonal differences between subjects collecting during weekdays and weekends (Broderick et al., 2004). Neither the AUCg on awakening (21 patients weekday-15 patients weekend) (Day 1, $z=-.961$, $p=0.34$; Day 2, $z=-.081$, $p=0.93$), or the AUCg for daily cortisol secretion were different between groups on either days (Day 1, $z=-.042$, $p=0.97$; Day 2, $z=-.064$, $p=0.95$). There were no differences on DHEA levels, or the ratio of Cortisol/DHEA on awakening or during the day.

Menstrual state

There was no difference between female patients who were pre- and post- menopausal on cortisol levels (12 patients pre and 14 patients post menopausal) There was no difference between female patients who were pre- and post- menopausal on cortisol levels (AUCg post awakening Day 1, $z=-1.312$, $p=0.19$; CAR Day 2, $z=-.114$, $p=0.91$; AUCg for daily cortisol secretion Day 1, $z=-.309$, $p=0.76$; Day 2, $z=-1.135$, $p=0.26$). DHEA levels (DHEA secretion on Day 1 following awakening $z=-1.777$, $p=0.07$), but not on Day 2 ($z=-1.347$, $p=0.18$). AUCg for daily DHEA secretion did not differ between groups (Day 1 $z=-1.066$, $p=0.29$; Day 2 $z=-1.663$, $p=0.09$), or the ratio of Cortisol/DHEA.

Physical illness

Secretion of DHEA following awakening as measured by the AUCg was lower in patients with physical illness (12 patients), defined as any form of chronic illness, including hypertension and diabetes, compared to those without (16 patients) (Day 1, $z=-1.929$, $p=.005$, patients with physical illness 60.4 (38.0) nmol/l vs patients without

physical illness 105.8 ± 59.7 nmol/l; Day 2, $z = -1.791$, $p = 0.07$, patients with physical illness 57.7 (34.6) nmol/l vs patients with no physical illness 114.7 (47.0) nmol/l). Secretion of DHEA was lower on Day 1 in patients with physical illness compared to those without ($z = -2.160$, $p = 0.03$, patients with physical illness 5.2 (4.2) nmol/l, patients with no physical illness 12.7 (5.2) nmol/l).

Time of awakening

There was no difference between early and late awakeners (18 early awakeners and 13 late awakeners on Day 1 (mean time 7:17) and 19 early awakeners and 13 late awakeners on Day 2 (mean time 7:17)), after performing a split on cortisol as measured by the CAR on awakening (AUCg Day 1, $z = -1.623$, $p = 0.10$; CAR Day 2, $z = -1.034$, $p = 0.30$), or the cortisol secretion during the day (AUCg Day 1, $z = -1.341$, $p = 0.18$; Day 2, $z = -.329$, $p = 0.74$). Similarly for DHEA no differences were found between the 2 groups (DHEA following awakening, Day 1, $z = -1.286$, $p = 0.19$; Day 2, $z = -.566$, $p = 0.57$; DHEA secretion during the day, measured by the AUCg (Day 1, $z = -1.640$ $p = 0.10$; Day 2, $z = .00$, $p = 1.00$). There was no difference on the ratio of Cortisol/DHEA following awakening (Day 1, $z = -1.041$, $p = 0.29$; Day 2 $z = -.350$, $p = 0.73$) or calculated as the AUCg during the day (Day 1, $z = -1.819$ $p = 0.07$; Day 2 $z = -.296$, $p = 0.77$).

Medication

No comparison was made between subjects who were taking SSRI's and noradrenergic antidepressants (tricyclics) due to small numbers.

Abuse

Below only significant results are reported (following correction for multiple comparisons). Furthermore, testing for numbers below 8 were not undertaken (for definition of abuse see Appendix 2). There was no difference between subjects who were exposed to a general traumatic event (adversity) early in life and those who were not exposed to adversity. Subjects that were physically abused (26 patients non abused 9 patients abused) had a higher DHEA following awakening on Day 1 ($z=-3.154$, $p<0.001$, abused 143.6 (52.9) nmol/l vs non abused 53.0 (38.6) nmol/l) and on Day 2 ($z=-2.785$, $p<0.001$, abused 116.3 (28.6) nmol/l vs non abused 64.3 (44.9) nmol/l). They also had a higher daily secretion of DHEA on Day 1 ($z=-3.375$, $p<0.001$, abused 16.05 (2.8) nmol/l vs non abused 7.5 (4.2) nmol/l).

BMI

There was no correlation between BMI and the CAR or DHEA following awakening or their daily secretion.

Age

There was an inverse correlation between age and the secretion of DHEA following awakening (Day 1, $r=-.557$, $p<0.001$; Day 2 $z=-.591$, $p<0.001$) and during the day on Day 1 only ($r=-.538$, $p<0.001$).

Gender

There was no difference in the daily cortisol secretion between genders (28 females-9 males) in subjects with acute depression (Day 1, $t=-1.230$, d.f.=30, $p=0.23$; Day 2, $t=-1.177$, d.f.= 28, $p=0.25$). Regarding the CAR (AUCg) post awakening there was no

difference between genders on Day 1 ($t=.376$, $d.f.=30$, $p=0.71$). Females had a higher CAR on Day 2 compared to males ($t=2.086$, $d.f.=30$, $p=0.05$).

Psychosis

Number for patients with psychosis were too small to undertake comparisons between psychotic and non psychotic patients during their current episode or with a lifetime psychosis.

Other variables in subjects with Treatment Resistant Unipolar Depression in remission

Smoking

No differences were found between patients who were smoking and those who were not (4 smokers and 12 non smokers) on cortisol levels (CAR AUCg post awakening, Day 1, $z=-.990$, $p=0.32$; Day 2, $z=-1.183$, $p=0.24$; AUCg for daily cortisol secretion on Day 1, $z=-1.175$, $p=0.24$ and on Day 2, $z=-.936$, $p=0.35$). Similarly there were no differences on DHEA levels (DHEA secretion on Day 1 following awakening, $z=-.816$, $p=0.41$ and on Day 2, $z=-1.291$, $p=0.19$; AUCg for daily DHEA secretion on Day 1, $z=-1.709$, $p=0.09$ and on Day 2, $z=-1.1715$, $p=0.09$), or the ratio of Cortisol/DHEA.

Place of collection

All patients undertook the test from their home. Therefore this variable was not assessed by undertaking a statistical comparison.

Day of collection

Between weekdays and weekends there were no differences on the AUCg post awakening (14 patients on weekdays vs 7 patients on weekends on Day 1 and 12 patients on weekdays vs 8 patients on weekends on Day 2) (AUCg Day 1, $z=-.965$, $p=0.33$; AUCg Day 2, $z=-.402$, $p=0.69$) and the AUCg for daily cortisol secretion on Day 1, $z=-.351$, $p=0.73$; Day 2, $z=-.653$, $p=0.51$).

There were no differences on DHEA levels (DHEA secretion on Day 1 following awakening $z=-.990$, $p=0.32$ and Day 2, $z=-.724$, $p=0.47$; AUCg for daily DHEA secretion on Day 1, $z=-1.234$, $p=0.22$ and Day 2, $z=-.870$, $p=0.38$), or the ratio of Cortisol/DHEA.

Menstrual state

There was no difference between female patients who were pre- and post- menopausal on cortisol levels (CAR Day 1, $z=-.667$, $p=0.50$; CAR Day 2, $z=-.293$, $p=0.77$; AUCg for daily cortisol secretion on Day 1, $z=-.500$, $p=0.61$ and on Day 2, $z=-.586$, $p=0.56$). All patients in group 2 undertook the test whilst at home.

Physical illness

No differences were found between patients who suffered from a physical illness and those without.

Time of awakening

There was no difference between early and late awakeners (8 early vs 9 late awakeners on Day 1 (time 7:15) and 10 early vs 6 late awakeners on Day 2 (time 7:15)) after

performing a split on the CAR (AUCg) (Day 1, $z=-.116$, $p=0.91$; Day 2, $z=-.118$, $p=0.91$) or the daily secretion of cortisol (Day 1, $z=-.735$, $p=0.46$; Day 2, $z=-.571$, $p=0.57$) or the secretion of DHEA following awakening (Day 1, $z=-.548$, $p=0.58$; Day 2, $z=-1.358$, $p=0.17$) or its secretion during the day (Day 1, $z=-.853$, $p=0.39$; Day 2, $z=-.980$, $p=0.33$).

There was no difference on the ratio of Cortisol/DHEA following awakening (Day 1, $z=-.426$, $p=0.67$; Day 2, $z=-1.176$, $p=0.08$) or during the day (Day 1, $z=-1.066$, $p=0.29$; Day 2, $z=-.296$, $p=0.77$).

BMI

There was a negative correlation between BMI and the CAR AUCg post awakening both on Day 1 ($r=-.471$, $p=0.05$) and Day 2 ($r=-.589$, $p=0.02$). There was a negative correlation with the BMI and the mean AUCg of 2 days of the ratio of Cortisol/DHEA ($r=-.833$, $p=0.02$).

Age

Regarding age no correlations were found with hormonal variables following correction for multiple comparisons.

Gender

There was no difference on the daily cortisol secretion between genders (18 females and 3 males) in subjects with remitted depression (Day 1 $t=-1.107$, $d.f.=17$, $p=0.28$; Day 2 $t=-.261$, $d.f.=14$, $p=0.79$). Regarding the CAR there was no difference between genders on Day 1 ($t=-1.419$, $d.f.=17$, $p=0.17$), or Day 2 ($t=-.561$, $d.f.=16$, $p=0.58$).

Psychosis

No differences were found between subjects with psychotic depression in remission and those with non-psychotic depression in remission.

Other variables in subjects with Treatment Resistant Bipolar Depression

Smoking

None of the subjects with Bipolar Depression was smoking therefore this comparison was not undertaken.

Place of collection

Regarding the CAR, there was no difference between subjects who collected on the ward (6 patients) or at home (11 patients) (AUCg post awakening Day 1, $z=-.067$, $p=0.95$; CAR Day 2, $z=-.804$, $p=0.42$; AUCg for daily cortisol secretion on Day 1, $z=-.474$, $p=0.63$ and on Day 2, $z=-.397$, $p=0.63$). Similarly there were no differences on DHEA levels (DHEA secretion on Day 1 following awakening $z=-.733$, $p=0.46$ and on Day 2, $z=-.333$, $p=0.74$; AUCg for daily DHEA secretion on Day 1, $z=-.340$, $p=0.73$ and on Day 2, $z=-1.532$, $p=0.12$), or the ratio of Cortisol/DHEA.

Day of collection

No differences were found between subjects who collected on weekdays and weekends (9 patients vs 6 patients) on the AUCg post awakening (Day 1, $z=-.1121$, $p=0.26$; Day 2, $z=-1.650$, $p=0.09$) and the AUCg for daily cortisol secretion (Day 1, $z=-.962$, $p=0.33$; Day 2, $z=-1.061$, $p=0.28$). There were no differences on DHEA levels, or the ratio of Cortisol/DHEA.

Menstrual state

The CAR was not different between female patients who were pre- (6 patients) and post- menopausal (4 patients) No cortisol differences were found between female patients who were pre- and post- menopausal (AUCg post awakening Day 1, $z=-1.732$, $p=0.08$, CAR Day 2, $z=-.853$, $p=0.39$; AUCg for daily cortisol secretion on Day 1, $z=-.655$, $p=0.51$ and on Day 2, $z=.00$, $p=1.00$). DHEA secretion post awakening (Day 1, $z=-1.640$, $p=0.10$, postmenopausal women 24.2 (31.9) nmol/l vs premenopausal women 116.4 (60.4) nmol/l, Day 2 AUCg $z=-1.938$, $p=0.05$, postmenopausal women 24.0 (29.7) nmol/l vs premenopausal women 109.5 (49.4) nmol/l) did not differ, nor the AUCg for daily DHEA secretion (Day 1, $z=-2.066$, $p=0.04$, postmenopausal women 5.5 (3.0) nmol/l vs premenopausal women 14.0 (4.4) nmol/l, Day 2, $z=-.745$, $p=0.46$, postmenopausal women 9.3 (4.3) nmol/l vs premenopausal women 10.5 (5.2) nmol/l). The ratio of Cortisol/DHEA was somewhat lower, but not statistically significant on Day 1 in postmenopausal women ($z=-1.768$, $p=0.07$, postmenopausal women 34.7 (21.3) vs premenopausal women 8.2 (5.7)).

Time of awakening

The CAR (AUCg) post awakening was not different between early (8 patients Day 1, 6 patients Day 2) and late awakeners (6 patients on Day 1 and 2) (median time 7:19 on Day 1 and 7:21 on Day 2 between early and late awakeners (AUCg Day 1, $z=-.081$, $p=0.93$; Day 2, $z=-.129$, $p=0.89$). The AUCg cortisol secretion was not different between groups (Day 1, $z=-1.486$, $p=0.13$; Day 2, $z=-2.195$, $p=0.03$, late awakeners 89.0 (19.7) nmol/l vs early awakeners 69.3 (11.2) nmol/l). Similarly there were no differences on DHEA following awakening (Day 1, $z=-.731$, $p=0.46$; Day 2, $z=-.406$,

$p=0.68$), or for the secretion of DHEA during the day (Day 1, $z=-.378$ $p=0.70$; Day 2, $z=-.586$, $p=0.56$).

Medication

No differences were found between subjects taking and not taking lithium, carbamazepine, lamotrigine, or antidepressants.

Abuse

Cortisol, DHEA and the Cortisol/DHEA ratio were not correlated with measures of psychological abuse, bullying or adversity in subjects with TRBD.

BMI

There was no significant correlation between BMI and the cortisol or DHEA following awakening or their daily secretion (data not shown).

Age

The expected negative correlation between age and DHEA daily secretion was not found (Day 1, $r=0.050$, $p=0.86$; Day 2, $r=-.503$, $p=0.06$).

A positive correlation was however found between age and the CAR on Day 1 ($r=0.602$, $p=0.02$).

Gender

There was no difference in the daily cortisol secretion (AUCg) between genders (10 females and 7 males) in subjects with TRBD (Day 1, $z=-.274$, $p=0.78$; Day 2, $z=-.976$,

$p=0.33$; Day 3, $z=-.447$, $p=0.65$; Day 4, $z=-1.155$, $p=0.25$), or the AUCg post awakening (Day 1, $z=-.775$, $p=0.44$; Day 2, $z=-.878$, $p=0.38$).

Comorbidity

None of the hormonal variables differed in patients with comorbid general anxiety disorder, panic disorder, agoraphobia or social phobia.

Psychosis

There was no difference in any of the main hormonal variables between patients with psychosis and those without. Of note is that only 4 patients with TRBD suffered with psychotic symptoms during their depressive episode.

The CAR (AUCg post awakening) was no different between groups without and with a history of psychosis on Day 1 ($z=-1.267$, $p=0.20$), or Day 2 ($z=-1.508$, $p=0.13$). 8 patients had a past history of psychosis in this group, but none was actively psychotic.

Comparisons between subtypes of Bipolar Disorder

BD Type I and Type II

Patients were split into those suffering from BAD Type I and Type II (as described by the DSM-IV) and were then compared. The AUCg post awakening did not differ between patients suffering from BAD Type I and II on any of the 2 days (CAR Day 1, $z=-.333$, $p=0.74$; CAR Day 2 $z=-1.464$, $p=0.14$). Secretion of Cortisol during the day was however different between the 2 main types of Bipolar Disorder on some of the days, an important finding (Day 2, $z=-2.488$, $p=0.01$, BD Type I, 9 patients median (IQR) 69.7 (14.1) vs BD Type II, 7 patients median (IQR) 84.5 (26.5); Day 4, $z=-2.309$,

$p=0.02$, BD Type I, 4 patients median (IQR) 60.0 (10.5) vs BD Type II, 4 patients median (IQR) 103.7 (78.9)). Secretion of cortisol did not differ between subgroups on Day 1 ($z=-1.372$, $p=0.17$, 6 patients with Type I BD, median (IQR) 67.0 (21.6) vs 5 patients with Type BD II, median (IQR) 78.5 (43.5)), or on Day 3 ($z=-1.443$, $p=0.15$, 4 patients with Type I BD, median (IQR) 79.4 (29.1) vs 4 patients with Type II BD, median (IQR) 100.2 (40.1)).

Overall on all 4 days secretion of cortisol was statistically lower on some days in Type I compared to Type II TRBD, although numbers were low for these sub-group comparisons.

Rapid Cycling Bipolar Affective Disorder

The CAR (AUCg) post awakening did not differ between rapid cyclers (6 patients) and non rapid cyclers (AUCg Day 1, $z=-.283$, $p=0.78$; Day 2, $z=-.453$, $p=0.65$). Secretion of cortisol during the day did not differ between groups on any of the days (Day 1, $z=-.663$, $p=0.51$; Day 2, $z=-.728$, $p=0.47$; Day 3, $z=-.333$, $p=0.74$; Day 4 $z=-.218$, $p=0.83$).

Other variables in patients with Treatment Resistant Bipolar Depression in remission

Smoking

Only 2 subjects were smokers, hence no statistical comparison was undertaken for this variable, due to the few numbers.

Place of collection

All but 2 subjects collected their samples whilst they were on the ward, hence no statistical comparison was undertaken for this variable, due to the few numbers.

Day of collection

Subjects that collected during weekdays and weekends were found not to differ in their cortisol secretion post awakening (9 patients vs 11 patients) (AUCg Day 1, $z=-.570$, $p=0.57$; CAR Day 2, $z=-.136$, $p=0.89$; AUCg for daily cortisol secretion Day 1, $z=-.434$, $p=0.66$ and Day 2 $z=-.325$, $p=0.74$).

Menstrual state

Between female subjects who were pre- and post- menopausal secretion of cortisol did not differ post correction on Day 1 (AUCg for daily cortisol secretion on Day 1, $z=-2.00$, $p=0.05$, premenopausal women 113.2 (28.6) nmol/l vs postmenopausal women 83.1 (15.5) nmol/l; Day 2, $z=-.940$, $p=0.35$, premenopausal women 107.0 (34.8) nmol/l vs postmenopausal women 80.5 (28.9) nmol/l). The AUCg post awakening was not different between subjects (Day 1, $z=-.192$, $p=0.85$, premenopausal women 1286.2 ± 388.3 nmol/l vs postmenopausal women 1291.5 (345.5) nmol/l; Day 2 $z=-.480$, $p=0.63$, premenopausal women 1146.4 (339.2) nmol/l vs postmenopausal women 1166.2 (376.7) nmol/l). There was no difference in the secretion of DHEA between pre- and post- menopausal subjects either following awakening (Day 1, $z=-1.461$, $p=0.14$, premenopausal women 111.1 (65.1) nmol/l vs postmenopausal women 37.6 (54.9) nmol/l; Day 2, $z=-.853$, $p=0.39$, premenopausal women 71.3 ± 112.5 nmol/l vs postmenopausal women 43.3 (39.3) nmol/l), or during the day (Day 1, $z=-.940$, $p=0.35$, premenopausal women 6.5 (11.0) nmol/l vs postmenopausal women 5.2 (3.4); Day 2 $z=-.745$, $p=0.45$, premenopausal women 7.2 (5.3) nmol/l vs postmenopausal women 3.9 (6.1) nmol/l).

The ratio of Cortisol/DHEA did not differ between these 2 groups on either Day 1 or 2 (Day 1, $z=-.313$, $p=0.75$, premenopausal women 337.2 (291.7) nmol/l vs postmenopausal women 235.3 (154.7) nmol/l; Day 2, $z=-1.043$, $p=0.29$, premenopausal women 206.4 (99.3) nmol/l vs postmenopausal women 340.1 (231.5) nmol/l).

Time of awakening

Early and late awakeners were compared by performing a split. The AUCg post awakening was not different between late (9 patients on both days) and early awakeners (9 patients on Day 1 and 8 patients on Day 2) (time 7:20 on Day 1 and 7:01 on Day 2) on either days (Day 1, $z=.145$, $p=0.55$; Day 2, $z=-.022$, $p=0.93$). Similarly secretion of cortisol during the day (AUCg) was not different between late and early awakeners on either Day 1 ($z=.217$, $p=0.47$), or Day 2 ($z=.044$, $p=0.89$). Early and late awakeners were compared by performing a split. The AUCg post awakening was not different between late and early awakeners on either days (Day 1, $z=-.653$, $p=0.51$; Day 2, $z=-.463$, $p=0.64$). Similarly the secretion of cortisol during the day was not different between late and early awakeners on either Day 1 ($z=-.370$, $p=0.71$), or Day 2 ($z=-.857$, $p=0.39$).

DHEA on awakening was not different between the 2 groups (Day 1, $z=-1.414$, $p=0.16$; Day 2, $z=-1.019$, $p=0.31$). Its secretion during the day also did not differ (Day 1, $z=-2.00$, $p=0.05$; Day 2, $z=-.342$, $p=0.73$).

Medication

Patients who were taking lithium, lamotrigine and antidepressants were not found to differ in their secretion of cortisol, DHEA or the Cortisol/DHEA ratio.

Abuse

Subjects who were exposed to a traumatic event early in life (adversity) did not differ in their cortisol or DHEA secretion. Comparisons for other forms of abuse were not undertaken due to the small number of subjects being exposed to abuse.

BMI

There was no significant correlation between BMI and the CAR or DHEA following awakening or the daily secretion of either cortisol or DHEA.

Age

Regarding age there was an inverse correlation with DHEA and its secretion following awakening (Day 1, $r=-.545$, $p=0.03$; Day 2 $r=-.670$, $p<0.001$), but not during the day (AUCg, Day 1, $r=-.260$, $p=0.39$; Day 2, $r=-.632$, $p=0.03$).

Gender

Cortisol post awakening was generally higher in female patients with remitted Bipolar Depression (AUCg Day 1, $z=-3.382$, $p<0.001$; females median (IQR) 1288.9 (721.9) nmol/l vs males median (IQR) 909.0 (189.6) nmol/l; Day 2, $t=.840$, d.f.= 16, $p=0.41$, 12 females mean \pm SD 1229.0 \pm 344.2 nmol/l vs 6 males mean \pm SD 1036.4 \pm 642.5 nmol/l; mean of 2 days, $t=2.661$, d.f.= 16, $p=0.02$, 12 females mean \pm SD 1345.1 \pm 228.0 nmol/l vs 6 males mean \pm SD 984.7 \pm 346.8 nmol/l).

Secretion of cortisol during the day did not differ between genders.

Other variables in controls

Smoking

There was no difference between smokers and non smokers on cortisol levels (AUCg post awakening Day 1, $z=-.395$, $p=0.69$; Day 2, $z=-1.386$, $p=0.16$; AUCg for daily cortisol secretion on Day 1, $z=-.412$, $p=0.68$ and on Day 2, $z=-.316$, $p=0.75$). Similarly there were no differences on DHEA levels (DHEA secretion on Day 1 following awakening, $z=-.00$, $p=1.00$ and on Day 2, $z=-.050$, $p=0.96$; AUCg for daily DHEA secretion on Day 1, $z=-.104$, $p=0.91$ and on Day 2 $z=-.672$, $p=0.50$), or the ratio of Cortisol/DHEA.

Place of collection

This comparison was not necessary in controls as all collection was undertaken from home.

Day of collection

Collection on weekdays as opposed to weekends did not affect the secretion of cortisol (AUCg post awakening Day 1, $z=-1.588$, $p=0.11$; Day 2, $z=-1.634$, $p=0.10$; AUCg for daily cortisol secretion on Day 1, $z=-1.381$, $p=0.16$; Day 2, $z=-.266$, $p=0.79$).

Time of awakening

Early and late time of awakening measured by performing a split between late and early awakeners did not have an effect on cortisol or DHEA secretion.

BMI

In controls BMI did not correlate with cortisol, DHEA or their ratio.

Age

Post correction no correlation was found between age and DHEA secretion in the morning. The ratio of Cortisol/DHEA positively correlated with age post awakening on Day 1 (Day 1, $r=.430$, $p=0.01$; Day 2, $r=.417$, $p=0.04$).

Gender

There was no difference in the daily cortisol secretion between genders in controls (Day 1, $t=.257$, $d.f.=41$, $p=0.80$; Day 2, $t=-1.018$, $d.f.=38$, $p=0.31$). Regarding the CAR there was no difference between genders on Day 1 ($t=1.292$, $d.f.=42$, $p=0.20$), or Day 2 ($t=1.869$, $d.f.=38$, $p=0.07$).

Table 27. Clinical demographics, comorbidity and types of medication. Patients with TRUD, TRBD and controls.

	<u>TRUD</u> <u>Mean SD or n(%)</u>	<u>TRBD</u> <u>Mean SD n(%)</u>	<u>HC</u> <u>Mean SD n(%)</u>
<u>Gender (%)</u>	28 F:10 M	10 F:7 M	26 F:21 M
<u>Age (years)</u>	52 (13)	52 (10)	50
<u>BMI (kg/m²)</u>	30 (7.3)	27.5 (6.2)	26.3
<u>Diagnosis</u>	TRUD	TRBD	-
<u>Comorbidity</u>	50% comorbidity Axis II PD (4) Eating Disorders (2) Anxiety Disorders (17) Past alcohol use (1)	47% comorbidity Axis II PD (1) Eating Disorders (1) Anxiety Disorders (5) Past alcohol use (1)	-
<u>Current Medication</u>	SNRI 12 SSRI 6 Tricyclics 8 Other antidepressants 12 2 or more antidepressants 10 1 mood stabiliser 16 2 or more mood stabilisers 5 Benzodiazapine/hypnotics 18 Antipsychotics 11 Clozapine 1 Thyroid hormones 8	SNRI 3 SSRI 0 Tricyclics 1 Other antidepressants 2 1 mood stabiliser 4 2 or more mood stabiliser 10 Benzodiazepine/hypnotics 4 Antipsychotics 0 Clozapine 1 Thyroid hormones 3	

F=Female, M=male, TRUD=Treatment Resistant Unipolar Depression, TRBD=Treatment Resistant Bipolar Depression, HC=Healthy Controls, BMI=Body Mass Index.

Table 28. Clinical demographics, comorbidity and types of medication. Patients with TRUD in remission, TRBD in remission and controls.

	<u><i>TRUD remission</i></u> <u><i>Mean SD or n(%)</i></u>	<u><i>TRBD remission</i></u> <u><i>Mean SD n(%)</i></u>	<u><i>HC</i></u> <u><i>Mean SD</i></u> <u><i>n(%)</i></u>
<u>Gender (%)</u>	18 F:3 M	14 F:6 M	26 F:21 M
<u>Age(years)</u>	54 (10)	52 (12)	50 (16)
<u>BMI (kg/m²)</u>	28.1 (7)	28.4 (6)	26.3 (4.5)
<u>Diagnosis</u>	TRUD	TRBD	-
<u>Comorbidity</u>	14% comorbidity Axis II PD (1) Eating Disorders (1) Anxiety Disorders (1)	0% comorbidity at time of assessment	-
<u>Current Medication</u>	SNRI 5 SSRI 1 Tricyclics 1 Other antidepressants 8 1 mood stabiliser 8 2 or more mood stabiliser 5 Benzodiazapine/ hypnotics 3 Antipsychotics 1 Thyroid hormones 0	SNRI 2 SSRI 2 Tricyclics 2 Other antidepressants 1 1 mood stabiliser 6 2 or more mood stabiliser 9 Benzodiazepine/ hypnotics 3 Antipsychotics 0 Thyroid hormones 5	

F=Female, M=Male, TRUD=Treatment Resistant Unipolar Depression, TRBD=Treatment Resistant Bipolar Depression, HC=healthy controls, BMI= Body Mass Index.

Further comparisons between subjects with Treatment Resistant Unipolar and Bipolar Depression

As described above, there were significant differences in the pattern of the CAR between the patients with TRUD and TRBD. In order to explore this further, additional analyses were undertaken.

Individual items of the HAM-D 21 Item were initially compared between groups. Groups of questions from the HAM-D 21 Item were then clustered together in an attempt to measure any potential differences in various symptoms between the 2 groups. This was because symptoms of sleep and appetite are measured by the HAM-D 21 Item in more than one question. Missing data are due to individual HAM-D questions sometimes not being filled out despite the overall data being used if the final HAM-D score was above cut off.

The aim of this was to compare differences between the symptoms of sleep, appetite and ultimately any differences between neurovegetative symptoms, in order to try to explain some of the cortisol findings, particularly following awakening. This was based on the the evidence that in Bipolar Disorder there is an increased likelihood of reverse neurovegetative symptoms compared to Unipolar Disorder (Perlis et al., 2006) and because of the evidence that patients with reversed neurovegetative symptoms may show a different neuroendocrine profile (Tsigos and Chrousos, 2002).

Sleep Disturbance

Individual sleep disturbances were compared (early insomnia, mid insomnia and early morning waking). Subjects did not differ with regards to initial insomnia ($z=-1.089$, $p=0.28$, 36 patients TRUD median (IQR) 1.5 (2.0) vs 15 patients TRBD 1.0 (2.0), mid insomnia ($z=-.989$, $p=0.32$, 37 patients TRUD median (IQR) 1.0 (1.0) vs 15 patients TRBD 1.0 (2.0)) and early morning waking ($z=-1.562$, $p=0.12$, 37 patients TRUD median (IQR) 1.0 (1.5) vs 15 patients TRBD 0.0 (1.0)).

When the two most “typical” sleep disturbance symptoms- (middle insomnia and early morning wakening – were added, this was more marked in unipolar patients than bipolar patients ($z=-2.22$, $p=0.03$, 35 patients TRUD median (IQR) 4.0 (2.0) vs 14 patients TRBD median (IQR) 3.0 (1.5)). When the total score of all three symptoms of sleep disturbance was calculated (total maximum score of 6), there was only a trend towards significance ($t=1.830$, $d.f.=50$, $p=0.07$; 37 patients with UD mean \pm SD 3.4 \pm 1.5 vs 15 patients with TRBD mean \pm SD 2.5 \pm 1.8).

Composite score of typical symptoms measured by the Hamilton

To measure whether the extent of typical symptoms is related to cortisol values, all 3 symptoms of sleep, appetite and diurnal mood variation worse in the morning were added (maximum score of 12) and compared between groups. Overall, patients with TRUD had higher scores than patients with TRBD ($t=2.341$, $d.f.=47$, $p=0.02$; 35 patients with TRUD mean \pm SD 4.9 \pm 1.8 vs 14 patients with TRBD mean \pm SD 3.6 \pm 2.0).

Composite score of atypical symptoms measured by the Hamilton

The above was also attempted in the reverse way and thus two atypical symptoms taken from the HAM-D 21 Item (diurnal mood variation worse in the evening and fatigue) were added and compared between groups. Although numerically lower in TRUD, there was no significant difference ($z=-.294$, $p=0.77$; 35 subjects with TRUD median (IQR) 1.00 (1.00) vs 14 subjects with TRBD 1.50 (1.25)).

Comparing the balance of typical and atypical symptoms within the Hamilton

A subtraction was then made between the 2 types of neurovegetative symptoms (the 3 ‘typical’ symptoms including 3 measures of sleep, 2 measures of appetite and diurnal mood variation) and of the 2 ‘atypical’ symptoms (reversed diurnal variation and fatigue) and the 2 groups were compared. The overall balance of typical to atypical symptoms assessed by the HAM-D 21 was higher in TRUD than TRBD ($t=2.156$, $d.f.=47$, $p=0.04$, 35 subjects with TRUD 3.4 ± 2.1 vs 14 subjects with TRBD 1.9 ± 2.5).

PSS

Data for perceived stress, as measured by the Perceived Stress Scale (PSS), were obtained in a subgroup of 25 subjects with TRUD (12 subjects with missing data) and 13 subjects with TRBD (4 subjects with missing data). Values were higher in subjects with TRBD compared to those with TRUD (33.6 ± 3.2 versus 26.7 ± 5.2 , $t=-4.345$, $d.f.=36$, $p<0.001$).

Life events

Life events were measured in 26 subjects with TRUD (11 subjects with missing data); they reported a median (IQR) of 2.0 (2.0) events in the preceding 12 months. Similar

data in 13 subjects with TRBD (4 subjects with missing data) found a median of 4.0 (3.0) events. The number of life events significantly differed between the 2 groups of subjects, being higher in patients with TRBD ($z=-2.890$, $p<0.001$).

Life events questionnaire was used for measurement of life events. This scale concentrates on measuring the events over the last 12 months. Childhood abuse was also evaluated from this questionnaire and the results are going to be evaluated in future. Patients with Bipolar Disorder have an excess of life events, which may be related to genetic factors (Hosang et al., 2010) as well as some of the characteristics of the condition such as impulsivity.

CHAPTER 4: DISCUSSION

STUDY I, TREATMENT RESISTANT UNIPOLAR DEPRESSION/PLASMA

For this pilot study, cortisol, DHEA and the Cortisol/DHEA ratio were measured in plasma in patients with TRUD and compared to controls. Patients were further divided into responders and non-responders to intensive optimised inpatient therapy and findings were compared between the two groups.

The first main finding is that treatment resistant depressed patients have both higher cortisol and higher Cortisol/DHEA ratios at baseline than controls, but unaltered DHEA levels. This is the only study to date to have specifically investigated DHEA and the Cortisol/DHEA ratio in patients with TRUD. Consistent with the prior literature on severe depression, in this study it is also shown that patients are markedly hypercortisolemic, when basal levels of cortisol are measured (Juruena et al., 2006).

No similar increase in the release of the other main adrenal steroid, DHEA, was found. If one were to accept that DHEA has an antiglucocorticoid activity as suggested (Bauer et al., 2002), then had DHEA also increased alongside cortisol there may not have been an excess of glucocorticoid activity. However, this study found that the ratio of Cortisol/DHEA is elevated, and that therefore there is likely to be an excessive “net” glucocorticoid activity in TRUD.

The second main finding is that in those patients with TRUD who went on to respond to inpatient treatment, DHEA levels were significantly lower on admission compared to

those who did not. Furthermore, the Cortisol/DHEA ratio was higher in treatment-responders compared to non-responders both on admission and on discharge.

Particularly striking was the magnitude of the difference in the Cortisol/DHEA ratio between responders and non-responders in the context of very similar basal cortisol levels in those two groups.

Since single cortisol measures are only of limited value in assessing the HPA axis (when compared to sequential sampling, dynamic or suppressive tests), it may be that there is more utility in the Cortisol/DHEA ratio on a single sample. Indeed, when DHEA was measured during the day in the group of healthy controls, there was no appreciable change in the Cortisol/DHEA ratio despite a significant diurnal change being apparent for cortisol. This finding agrees with previous observations where it was found that DHEA plasma levels covary with cortisol levels (Rosenfeld et al., 1975). Thus, it is possible that the Cortisol/DHEA ratio is a more accessible and stable measure than cortisol alone, which could heighten its utility in future clinical research.

The concept of using a ratio in medicine is not novel. There are other fields in medicine where in various conditions a ratio of different variables is used in order to define the condition, such as polycystic ovarian syndrome, dyslipidaemias and in those at risk of cardiovascular disease. Nevertheless, ratios do not replace the importance of understanding the components of that ratio; different factors of importance may affect differentially cortisol, DHEA and their ratio and we do not yet fully understand the impact on the Cortisol/DHEA ratio of different perturbations of the HPA axis. Thus, the ratio may remain stable when there are large changes in both the underlying cortisol and/or DHEA values. Whilst this stability is a potential strength of the use of this ratio,

and data suggest that the ratio may be more stable through the day than individual cortisol values, more work needs to be done to clarify the relationship between the ratio and the underlying state of the HPA axis.

It is difficult to interpret the relationship between response to treatment and the Cortisol/DHEA ratio: whilst as a whole patients with resistant depression have a raised Cortisol/DHEA ratio, those who then respond to treatment are the ones with the higher Cortisol/DHEA ratios, i.e. more abnormal biological parameters. One interpretation is that treatment resistant depression is not a unitary concept, and that such patients include not only those with a biologically driven treatment resistance – i.e. a more severe form of the HPA axis dysfunction seen in depressive illness as a whole – but also those whose treatment resistance relates to other factors such as personality, trauma, social circumstances and familial or occupational dynamics. Given that the main therapeutic intervention received by patients during inpatient stay is a largely biological one – with the use of multiple psychopharmacological interventions – then one might hypothesise that those most likely to respond would be those with a more biological illness. However, treatment did not lead to a normalisation of the HPA axis dysfunction even with response: the Cortisol/DHEA ratio remained elevated in treatment responders even on discharge. Nevertheless, such findings are not unusual in studies looking at treatment effects in depression; for example, these results parallel those of the original studies in this area using the dexamethasone suppression test that found that DST non-suppressors generally responded more favourably to biological than psychological treatments, and that DST non-suppression often persisted after clinical response (albeit with a higher risk of subsequent relapse and/or suicide) (Task force on laboratory tests in psychiatry, 1987).

There was no evidence that patients in whom axis-II difficulties were present had a different biological profile in terms of the measures taken in this study, although the sample size with a comorbid axis-II disorder was small and the observed numerically but not statistically lower Cortisol/DHEA ratio on admission in those with axis-II disorders needs further investigation in larger samples. Indeed, this was tested further, using salivary measures, in studies II and III in this thesis.

Furthermore the fact that the BMI of most patients as well as controls was in the overweight range could also have influenced the hormone levels that were measured, since most previous studies have shown higher levels of both cortisol and DHEA in subjects that have a high BMI (Maccario et al., 1999; Svec and Shawar, 1997; Ukkola et al., 2001). However, as BMI did not differ significantly between patients and controls, this does not explain differences in cortisol and DHEA.

This study was an initial pilot study, with several limitations. Blood samples were collected on one day only; although many prior studies have used a similar methodology, more reliable results may be obtained by collecting over a number of days. Second, although the time of sample collection was standardised, the time of awakening of the patients was not, and subjects tested soon after waking may have had artificially high cortisol values due to the stimulatory effect of awakening on cortisol release. This effect however is primarily seen in saliva rather than the plasma collections.

The numbers in the study were adequately powered for the main analyses, but are somewhat small for sub-analyses. In addition, the multiple testing in the sub-analyses lends caution to those findings.

Another potential criticism is that use of medication was not controlled for, given that it was felt unethical to enter subjects into a substantial washout period for the purposes of this study. While medication could have affected the comparison between patients and controls, it is unlikely to have affected the comparisons between treatment responders and non-responders as they were taking similar doses and types of medication on admission. Furthermore, by undertaking statistical comparisons there was no detection of an influence of mood stabilisers, thyroid hormones or antipsychotics on cortisol or DHEA values.

There were no significant differences in gender to account for the differences in the results, despite the modest sample size, especially when subjects were divided into responders and non-responders. Menstrual cycle was not controlled for since many prior studies have suggested that basal activity of HPA is not influenced by the stage of the menstrual cycle (Parry et al., 2000; Groschl et al., 2001; McCormick and Teillon, 2001; Cevik et al., 2004), as opposed to stress induced activity (Kirschbaum et al., 1999; Roca et al., 2003), which can differ depending on the phases of the cycle (Kirschbaum et al., 1999; Roca et al., 2003). In this sample there were no differences between subjects that were pre- and post-menopausal.

Finally, it is possible that factors other than treatment or treatment response that were not controlled for could have affected results, such as seasonal variation. The season of

testing was broadly similar in those tested before and after treatment, but one cannot exclude such an effect on the comparisons before and after treatment. It is accepted that in winter there is hypersecretion of cortisol due to hyperarousal and high stress levels and also due to the inhibitory influence of the SCN on the periventricular nucleus of the hypothalamus, as daylight leads to a stimulation of the SCN nucleus (King et al., 2000; Weitzman et al., 1975).

In summary, this study provides further evidence of a disrupted HPA axis in TRUD, and extends previous research by demonstrating that the ratio of Cortisol/DHEA is also abnormally elevated. These findings were shown on one day only. Unlike cortisol alone, the Cortisol/DHEA ratio seems to be an important clinical biological marker that can potentially predict response and non-response to treatment. This raises the possibility of targeting more aggressive pharmacotherapy for patients that on admission exhibit such results, potentially leading to faster resolution of symptoms.

STUDY II and III, TREATMENT RESISTANT UNIPOLAR AND BIPOLAR DEPRESSION /SALIVA

Short summary of main findings

The most important findings from these studies are that a) subjects with TRBD have a lower CAR compared to TRUD and controls; b) subjects with TRUD have a higher CAR than TRBD and controls; and c) that during remission there is normalisation of the HPA axis in both TRUD and TRBD, showing that effective treatment is key in these conditions. The salient findings in different groups are presented in this chapter.

The lower CAR in TRBD is a finding that is presented for the first time. This was prominent during the first day of testing, possibly related to novelty, and will be discussed in detail in this chapter.

Cortisol

Comparison of patients with Treatment Resistant Unipolar Depression and controls **CAR**

Two main methods of measuring cortisol and HPA axis activity were used in this study. The primary one was the CAR and the other was the secretion of cortisol throughout the day. The CAR was calculated by the AUC_g and the AUC_i. The AUC_g was significantly higher in patients compared to controls when measured over 2 days adding to the body of evidence that there is hypercortisolemia in depression. The AUC_g has been previously shown to associate with the diurnal cortisol profile (Edwards et al., 2001). This hypercortisolemia related to the CAR has never been shown in TRUD, although it has been previously shown in uncomplicated Unipolar Depression (Bhagwagar et al., 2005). Therefore it is shown here for the first time that basal hypercortisolemia, as shown by the AUC_g and the secretion of cortisol during the day, is not only present in subjects with depression (Stetler and Miller, 2011), but also specifically in TRUD. It should be mentioned that, in the aforementioned seminal paper, Bhagwagar and colleagues found that the CAR was higher in 20 medication free patients with depression compared to controls (Bhagwagar et al., 2005).

In contrary to the above, the AUC_i, related to the sensitivity of the system (Edwards et al., 2001; Chida and Steptoe, 2009) was not higher in patients than controls. The AUC_i

has never been previously measured in TRUD and possibly in uncomplicated Unipolar Depression. The fact that it was not different in patients compared to controls on any of the days measured, in the setting of a higher AUC_g, suggests that in TRUD there is basal hypercortisolemia but a normally responsive HPA axis. This finding is similar to that of Juruena and colleagues (2006) who found hypercortisolemia in TRUD, but a normal suppressive response to prednisolone. However, the contrast with TRBD, where there is both a lower AUC_g and AUC_i, suggests that the HPA axis may be both hypocortisolemic and under responsive in TRBD, and by extension that the way these patients respond to stress is potentially different. Furthermore of interest is that a recent study showed that stressful life events were lower in childhood in patients with BD compared to controls. After their first episode subjects with BD had again less stressful life events compared to controls (Horesh et al., 2011).

A delay in the increase of cortisol levels post awakening was observed in the study. This is not the first time that such a delay is observed. For example Argyropoulos and colleagues observed a relative delay of the cortisol response (peak at 20 min post inhalation) following 35% of CO₂ inhalation (Argyropoulos et al., 2002). A similar delayed response is also seen with insulin challenge (Aardal-Eriksson et al., 1998). It is not clear at present as to why this happens.

Daily cortisol secretion

In line with the above, daily cortisol secretion was also found to be higher in TRUD compared to controls on Day 1, Day 2 and Day 3, again showing that these patients exhibit basal hypercortisolemia when daily cortisol secretion is measured in saliva over 4 consecutive days (in 3 out of 4 days). One explanation of the null finding in Day 4 is

that this may be related to the smaller numbers on Day 4 as described in Chapter 3.

Alternatively it may be that there is not complete dysregulation of the HPA axis in TRUD. Given that basal HPA activity consists of secretory and refractory phases, some stressful stimuli may have occurred during the secretory phases of the activity therefore producing a response and others may have occurred during the refractory phase, thus failing to produce a response (Argyropoulos et al., 2002). This may have become more apparent in our experiment as testing occurred over multiple days.

Cortisol concentrations

Basal hypercortisolemia has been previously shown in TRUD when single salivary cortisol concentrations were measured in the morning, noon and evening (Jurueña et al., 2010). In the current study we found that cortisol was higher at noon and in the evening on Day 1 and depressed patients had higher cortisol levels at noon on Day 2. Thus, it seems that there is dysregulation of the HPA axis, which is exhibited as hypercortisolemia at different time points of the day over different days. One explanation of the increased cortisol levels over some days only and at specific time points is that the dysregulation of the HPA axis is not complete. Given that there is pulsatile 24 hr hormone secretion of cortisol, some of the abnormalities we found may be related to gaps in its basal regulation. That however does not preclude that dysregulation is complete throughout the 24 hr period, which is shown by an irregular pattern of endocrine abnormalities. This irregular pattern of basal cortisol secretion may be even related to stressful events at specific time points. The sympathetic system and splanchnic sympathetic activation may be responsible for this, as impaired negative feedback in depression may not be the only reason behind basal hypercortisolemia (Carroll et al., 2011). In the current study, although various methods were used to

record stressful events it only makes sense for one to appreciate that this may not always be totally accurate, as it also depends on the subject undertaking the experiment to record stressful life events accurately and understand what is stressful for them.

Summary

In summary, we found basal hypercortisolemia in patients with TRUD compared to controls, as measured both by the salivary CAR (AUCg) and the daily secretion of cortisol over 3 consecutive days.

Thus, we were able to replicate and extend the finding of the pilot study, by using salivary measurement of cortisol, a reliable, more ‘patient acceptable’ way of repeatedly assessing hormonal levels during the day. This adds to the emerging body of evidence that there is hypercortisolemia in TRUD, and suggests that TRUD falls into the sub-categories of depression in which there are more biological abnormalities, such as psychotic and melancholic depression. As previously mentioned, patients with TRUD have also been shown to have other HPA axis abnormalities, such as non suppression to the DST (Juruena et al., 2006). In future, 24 hr salivary studies over multiple days may shed light as to whether there is complete basal hypercortisolemia in TRUD, or if this is a partial phenomenon possibly exacerbated by stressful events. Furthermore measurement of cortisol in hair, which reflects cortisol levels over a few months as opposed to the date taken may be a better way to measure cortisol in future studies.

Comparison of patients with Treatment Resistant Unipolar Depression in remission and controls

CAR and daily cortisol secretion

The CAR (both AUC_g and AUC_i) did not differ between patients with remitted TRUD and controls. Similarly secretion of cortisol during the day was not different between groups.

It has previously been demonstrated that in patients with depression who have a hyperactive HPA axis, there is a normalisation of the HPA axis following remission (Holsboer et al., 1987; Pariante et al., 1997; Zobel et al., 2001). Although this normalisation of the HPA axis hyperactivity is seen in parallel with clinical remission, it is not necessarily related to antidepressant use (Ising et al., 2005). However, treatment with some antidepressants has been shown to decrease salivary cortisol (Scharnholz et al., 2010). The aforementioned study showed that afternoon salivary cortisol (1600 hrs) was lowered over 4 weeks of mirtazapine treatment in depressed subjects. Interestingly this was not different between responders and non responders. Different antidepressants, such as venlafaxine did not seem to have the same effect on cortisol in the same group of patients. Antidepressants in our study on the other hand did not have an appreciable effect on cortisol (possibly related to small numbers).

Another way to look at this is that patients in full remission from a depressive episode have no residual biological abnormalities, although previous studies have shown that in TRUD this is not always the case (Juruena et al., 2006). Secretion of cortisol has been previously measured in subjects with depression and in remission. Using the DEX/CRH test it has been shown that abnormalities in the HPA axis not only persisted in patients

at high risk of relapse (Aubry et al. 2010; Ising et al., 2007), but also predicted relapse (Zobel et al., 2001).

Summary

In summary, our findings show that patients with TRUD in remission have a normalised HPA axis activity, which agrees with some but not all of the previous literature in non-resistant depression also showing that there is normalisation of the HPA axis following treatment. This finding may also link with recent MRI data comparing hippocampal volume in patients with remitted depression and controls, which showed no significant difference (Kempton et al., 2011). This meta-analysis also showed that hippocampal volume is smaller in depressed patients compared to controls, suggesting that hippocampal atrophy also reverses with treatment. Given the hypothesis that it is high cortisol levels that may cause hippocampal atrophy in the acute depressive state, then these two sets of findings fit well together.

Comparison of patients with Treatment Resistant Unipolar Depression and those in remission

CAR (AUC_g)

Patients with TRUD have a higher CAR compared to remission on Day 1, Day 2 and once the mean of the 2 days was calculated. This is a similar finding to that when patients with TRUD and controls were compared.

AUC_i

The AUC_i was not different in subjects with TRUD compared to those in remission on Day 1, Day 2 and once the mean of the 2 days was calculated.

Daily cortisol secretion

Cortisol secretion during the day was higher in patients during the depressive phase of their illness compared to remission on Day 1, but not different during the other days.

Summary

In summary, we did find that patients during a depressive episode have a higher cortisol secretion during the day and a higher morning CAR (AUCg) compared to those in remission. This is the first time that a normalisation of an abnormal CAR has been shown in remitted TRUD, although a reduction in basal hypercortisolemia has previously been shown (Jurueña et al., 2010). Although it is possible that the remitted and acutely ill groups differed in other aspects than just the remission of depression, we were not able to find any significant differences that might account for a different CAR. Furthermore, 8 patients undertook the test both during remission and in the acute phase of their illness, giving a partial overlap of patients in both groups. This finding also agrees with our previous findings in the pilot study between remitted TRUD patients and controls and with some previous research showing normalisation of the axis following remission as discussed earlier.

Not all studies in non-resistant depression have found that the axis normalises with remission, as in some studies the CAR has also been found to remain elevated in remission (Bhagwagar et al., 2003). This may be due to the fact that sample characteristics of patients are different, such as inpatients vs outpatients, duration of illness ie. chronically unwell patients vs acute depression, patients with and without psychosis, other comorbidities such as anxiety and PTSD and personality disorders, all of which could affect the results. It may also be that the assessment in some studies was undertaken immediately upon remission, by which time cortisol levels have not yet

normalised, or that those patients with an elevated CAR on remission are at higher risk of relapse. It should also be of note that in our pilot study we did not find normalisation of the HPA axis in responders. Thus, it is possible that the above normalisation of the axis found in this sample is not a stable finding, or that subjects with other types of depression do not show resolution of the axis following remission. For these reasons, it is important that further studies are undertaken in order to draw conclusions on cortisol in remitted depression, including long term follow up studies.

Comparison of patients with Treatment Resistant Bipolar Depression and controls **CAR**

Perhaps the most important finding in this thesis is that patients with TRBD have a lower CAR (AUC_g and AUC_i) on Day 1 compared to controls. It is the first time that patients with Bipolar Depression have been shown to have a lower CAR compared to controls and indeed the first time that the CAR has been measured in Bipolar Depression, or Bipolar Disorder (Type I or Type II), during any acute illness phase. Furthermore the CAR has never been measured in TRBD, which may carry even more severe biological abnormalities related to it being a condition, with early onset. This is supported by recent evidence, which shows that patients with Bipolar Disorder have an earlier age at onset of the condition and earlier age at onset of their first major depressive episode compared to Unipolar Disorder (Souery et al., 2012). It may also be that TRBD has an earlier onset than BD in general, although this hypothesis is yet to be tested.

On a slightly different note, in the current study, we differentiated between the 3 phases of Bipolar Disorder, and only included patients during their depressive phase, as

opposed to amalgamating different illness phases, which may have masked underlying hormonal abnormalities. Thus, only bipolar depressed patients were included (by applying very stringent inclusion criteria and ensuring, following examination, that mixed cases were excluded). However, taking into account that the CAR was measured on 2 days only, one needs to keep an open mind and be aware of the possibility of Type II error.

Previous studies (see Chapter 1), which measured basal cortisol levels, by including patients in remission and in both acute phases of their illness, have shown basal hypercortisolemia. Hypercortisolemia has been previously shown in mania (Linkowski et al., 1994). These previous results are difficult to interpret, as they hypothesise that Bipolar Disorder is a homogeneous condition, and that all phases of the illness carry the same underlying biological abnormalities. This is problematic; even historically, mania and melancholia have been described as distinct diseases with separate etiologies, from ancient times by Homer (description of Ajax and his death by suicide in Iliad), Hippocrates, Aretaeus of Cappadocia and Soranus of Ephesus. Even within the depressive illness phases, the current study showed that in Type I Bipolar Disorder, the CAR is lower compared to Type II Bipolar Disorder on Day 1, albeit that there is a modest sample size for this comparison.

Effect Size

The Effect Size on Day 1, calculated as the difference in means between patients and controls divided by their SD was found to be 0.6, which is considered to be large, according to the Cohen criteria (Cohen, 1988). Although the CAR did not differ when the mean of the 2 days was calculated between groups (the CAR was not statistically

different in patients with Acute TRBD and in controls), the ES of the CAR was large only on Day 1. This is relatively surprising given that the CAR is thought to be a stable marker especially over 2 days (Wust et al., 2000; Edwards 2001). The CAR has been shown to be highly hereditary based on twin studies (Wust et al., 2000). The difference in the CAR in our study could be related to the role of the hippocampus and novelty in this disorder, as discussed later in this chapter.

The CAR was not statistically different between the 2 days. Furthermore the subjects that took part in the study on both days were the same.

Explanation of low CAR in subjects with Treatment Resistant Bipolar Depression on Day 1.

Chronicity

A possible explanation of the low CAR on Day 1, is that subjects with TRBD are more prone to stress, or that their HPA axis has been downregulated in response to chronic stress. Previous studies have found that compared to Unipolar Disorder, HPA axis dysfunction is greater and more persistent in Bipolar Disorder (Daban et al., 2005), and that the severity of the manic episodes is associated with the degree of HPA axis dysfunction (Daban et al., 2005). Bipolar Disorder has an earlier age of onset compared to Unipolar Disorder (Kessler et al., 2007). In our sample, 2/17 patients (12%) with Type I Bipolar Disorder committed suicide following their discharge. Although higher mortality rates do not necessarily imply greater severity, this outcome is different (so far), compared to the outcome of our TRUD group, of which none of the subjects has committed suicide (0%).

Previous research has shown that a lower morning cortisol response is associated with a more severe and chronic course and worse illness clinical outcome in depression and that chronic and severe stress is associated with a blunted CAR (Meinlschmidt and Heim, 2005; O' Connor et al., 2009).

Chronicity of stress has been found to downregulate the HPA axis in other psychiatric disorders. For example abuse chronicity has been inversely correlated with the salivary AUCg (CAR) in sheltered battered women with PTSD (Johnson et al., 2007). In this study, the CAR was found to be lower in PTSD victims, only when chronicity of the abuse was taken into account (in more chronic compared to less chronic cases). Not just in human studies, but in experiments in mice, chronic stress exposure leads to hypocortisolemia in certain types of mice that lack the lysophosphatidic acid (LPA) receptor pathway, which plays an important role in adult hippocampal neurogenesis (Castilla-Ortega et al., 2011). Thus, it may be that subjects that are genetically susceptible to stress develop hypocortisolemia following chronic stress exposure. Bipolar Disorder is a chronic condition with earlier age of onset as well as earlier age of depressive episodes, which may explain the difference in our findings (hypocortisolemia as opposed to the hypocortisolemia in TRUD), which in conjunction with novelty as an additional stress may have led to the low CAR on Day 1.

Daily stressors

Another interesting point is that the *magnitude* of the CAR, may be related to anticipation of upcoming demands, which may be essential for its regulation. In practice this may mean that the CAR may be higher in subjects, where there is anticipation of an event of some sort.

The importance of single stressors in regulating the CAR has been illustrated in a study by Rohleder and colleagues (Rohleder et al., 2007). In the aforementioned study, also described in Chapter 1, participants with no psychiatric diagnosis showed an increased CAR on the morning of a competitive ballroom dance tournament, whereas their CAR on the previous training day remained normal. Although these subjects had no psychiatric diagnosis, this study's importance lies in the fact that it unravels the impact of a single stress stimulus on the CAR response. Interestingly, in our study, the CAR was found to be relatively higher in controls on Day 1 compared to Day 2 (although not statistically different between the 2 days). Thus, in line with the above hypothesis, the anticipation of taking part in the study may have contributed to a higher CAR on Day 1 in patients with no psychiatric diagnosis, in line with the above study by Rohleder and colleagues. This may be a normal process, which is not found in patients. Thus, this may be a consequence of the fact that controls do not show this dysregulated HPA axis response, as opposed to patients.

Novelty of stimulus

Novelty seeking is another important factor that might determine the magnitude of the CAR. However for a normal CAR, a minimum level of hippocampal integrity may be required and it seems that only above that threshold there is a positive association between the CAR and the function of the hippocampus. The opposite may also be true, that is, a negative association may exist between hippocampus and the CAR as may have happened in our experiment. This may be related to the genetic profile of Bipolar Disorder. The hippocampus has been previously shown to cause inhibition of the HPA axis (Herman et al., 1996; Jacobson and Sapolsky, 1991; Herman et al., 2005).

Hippocampal stimulation decreases GC secretion in rat and human (Dunn and Orr,

1984). Hippocampal lesions increase ACTH release and PVN CRH levels (Herman et al., 2005). Therefore the hippocampus seems to be involved in inhibiting HPA axis responses, to at least some forms of stress. Furthermore as also mentioned earlier in the chapter in arrhythmic animals the SCN exhibits an even greater inhibitory input in target sites as the hippocampus.

The hypothesis that novelty may have a impact on the magnitude of the CAR and that this is related to the day of testing, was tested by Thorn and colleagues, in which the CAR was found to be higher on Day 1, when measured over 2 days (Thorn et al., 2009). Indeed this was observed when authors looked at their past databases. Novelty would be expected to be more prominent on Day 1 of an experiment. However, this effect is not always seen in studies. In our study, novelty may have caused the opposite abnormality, that is, a much lower rather than higher CAR on Day 1 compared to Day 2. In our sample the dysregulation of the HPA axis, may have been translated as more extreme downregulation on Day 1, which was due to novelty the most stressful day of the protocol.

The fact that these patients differ regarding the novelty effect might also be explained if we take into account the BIS (Behavioral Inhibition System) and BAS (Behavioral Approach System) model. According to the BAS dysregulation model, bipolar spectrum disorders stem from hypersensitivity of a behavioral-motivational system, the Behavioral Approach System (BAS), which facilitates approach to rewards and safety cues in active-avoidance paradigms (Alloy and Abramson, 2010). Individuals with bipolar disorders are hypothesized to be hypersensitive to reward-relevant cues (i.e., both opportunities to gain rewards and negative cues of failure to obtain/loss of

rewards). This hypersensitivity leads to extreme shifts in BAS activation, with hypomania/mania reflecting extreme approach behaviors (i.e., BAS hyperactivation) and depression reflecting extreme shutdown of approach behaviors (i.e., BAS hypoactivation). Although previously thought that low BIS and high BAS predict more severe forms of Bipolar Disorder and often progression to Bipolar I Disorder, this hypothesis was consequently rejected by Alloy and colleagues who found that both high BIS and high BAS predict more severe forms of BD (Alloy et al., 2012). One particular aspect of the BAS, the fun seeking model is very prominent in Bipolar II and Bipolar I Disorder. Whereas BAS Drive assesses persistence in goal striving and BAS Reward Responsiveness assesses responsiveness to obtained rewards (or consummatory, postgoal positive affect), the BAS Fun-Seeking subscale assesses the component of BAS sensitivity relating to willingness to approach *new* and potentially rewarding experiences (e.g., “I’m always willing to try something new if I think it will be fun” and “I crave excitement and new sensations”). This is also the subscale most related to impulsivity, mostly prominent in Bipolar I Disorder. Thus the novelty effect can only be seen in this particular type of Affective Disorders, Bipolar Disorder, as opposed to Unipolar Disorder, which may be another reason why the CAR was different on Day 1 only on this subgroup of patients.

Summary and implications

In summary, we found that patients with TRBD have a lower CAR measured by both the AUCi and AUCg compared to controls. This is a novel finding that if replicated will have important implications in the understanding of the neurobiology of Bipolar Disorder.

Measurements of the CAR on more than 2 days may be more informative regarding HPA axis abnormalities, and could help determine the extent to which novelty may be implicated in the observed findings. It may also be that fluctuations in the extent and/or direction of change of HPA axis dysregulation are part of Bipolar Disorder, something that again cannot be ruled out unless the CAR is measured over more extended periods of time. Support for this contention of a variable change in the HPA axis, comes from a previous study by Deshauer and colleagues, which demonstrated that when using the DST, there was only intermittent cortisol non suppression during monthly sampling in Bipolar Disorder (Deshauer et al., 1999). However if novelty is indeed implicated on the lower CAR on Day 1, it is unlikely that the CAR will be lower on days other than the first. This may have implications in terms of how bipolar depressed patients respond to novel stressors in daily life.

Finally, the hypo responsiveness of the axis in TRBD may have been observed on Day 1 randomly. In order to elucidate whether this is the case measuring the CAR over more than 2 days may be required.

Further interpretations of findings of this study

Although in TRUD both the CAR (AUC_g) and the secretion of cortisol during the day were higher in patients compared to controls, when patients with TRBD were compared with patients with TRUD, only the CAR (AUC_g and AUC_i) differed, but the daily cortisol secretion was not different. This raises questions regarding the similarity of the 2 conditions and especially the structures implicated in their genesis. For example it

may be that, since the CAR is neurally regulated by the SCN and the hippocampus, these structures are implicated in the aetiology of TRBD. The SCN has been previously implicated in Bipolar Disorder. For example disruption of one of the core proteins in the master circadian clock can trigger mania-like behaviours (Roybal et al., 2007). This, as well as other clinical characteristics of the condition related to seasonal factors, travelling and photoperiod (light) exposure, which are linked to Bipolar Disorder, may make the SCN a core structure implicated in the condition. Seasonality, defined as a tendency to experience seasonal variations in mood, behaviour and vegetative functions, can affect 20-25% of patients with Bipolar Disorder, with some studies showing that relapse of depressive episodes is more common in the winter and autumn (Kim et al., 2011). Some studies showed that sleep time in Bipolar Disorder is less in the summer compared to the winter (Kim et al., 2011).

It is not entirely clear at the present time how the SCN affects the hippocampus. Recent research, however, suggests the following:

1. A functional circadian system is required in hippocampal-dependent learning (Ruby et al., 2008). To test this hypothesis, the authors used a novel object recognition task (a hippocampal mediated task) in rhythmic and arrhythmic hamsters. Arrhythmic animals performed poorly on this task and could not detect the novel object. This learning performance was restored by the gamma-aminobutyric acid (GABA) antagonist pentylene-tetrazol (PTZ). Thus, lower GABA, the primary neurotransmitter of the SCN, restored the altered pattern in these arrhythmic animals, which was altered so as to increase the inhibitory input at SCN target sites involving cognition and novelty in the hippocampus. The resulting elevations of

inhibitory tone in SCN targets reduced synaptic excitability in the hippocampus and attenuated the ability to gather and consolidate information. Interestingly, in an analysis of 1868 cases of Bipolar Disorder, one of the most strongly related polymorphisms lay within a gene encoding one of the subunits of the GABA_A receptor (Breuer et al., 2011).

2. In rodents, winter-like photoperiods impair spatial learning and alter hippocampal plasticity (Pyter et al., 2005; Pyter et al., 2006).

Despite this accumulating evidence, it is not clear whether circadian rhythm disturbances are the underlying causes of Bipolar Disorder, and thus more research is needed possibly in early phases of this condition and in subjects at risk of BD (prospective studies) as well.

Comparison of patients with Treatment Resistant Bipolar Depression, Treatment Resistant Bipolar Depression in remission and controls.

The CAR, measured by the AUC_g and AUC_i was not different between remitted patients and controls. Similarly, patients in remission had a normalised HPA axis response compared to acutely depressed TRBD.

The CAR has been previously measured in subjects with remitted Bipolar Depression and in the offspring of patients with Bipolar Disorder. For example, a study by Deshauer and colleagues (Deshauer et al., 2006) measured the CAR in patients with remitted Bipolar Disorder who were stable on lithium medication and in the offspring of patients with Bipolar Disorder and found that the CAR did not differ between these 2 groups.

To assess whether cortisol abnormalities are a state or a trait marker of Bipolar Disorder, the majority of the studies have concentrated on measuring basal and dynamic cortisol levels in the offspring of patients with Bipolar Disorder (Ellenbogen et al., 2002; Ellenbogen et al., 2006). These studies have shown a degree of hypercortisolemia in the offspring of patients with Bipolar Disorder, during the day. Since these studies are rarely prospective, it is not easy to disentangle whether this heightened sensitivity to stress is the cause of more stressful events, or whether the higher amount of stress and life events in these individuals causes the HPA system to become more sensitive, as it is possible that growing up in a stressful environment with mentally ill parents affects cortisol levels. This stressful environment, or an exaggerated response to stress, may be part of the underlying reason why the HPA axis becomes sensitive, or downregulated with time. Adolescent patients do not often show the same abnormalities as in adulthood, as also shown by a recent meta-analysis which showed that adolescent patients with Unipolar Disorder do not show the typical hippocampal volume reduction seen in adulthood and old age (Kempton et al., 2011).

It is also possible that non normalisation of the HPA axis is explained by incomplete remission, as complete recovery between episodes is not always the case in Bipolar Disorder (Angst et al., 2000).

Our results however show that dysregulation of the HPA axis is a state marker of TRBD and that with adequate therapeutic treatment and in remission there are only few abnormalities on the daily cortisol secretion.

Summary

In summary, our findings show that patients in TRBD in remission have a normalised HPA axis activity, similarly to patients with TRUD in remission. It is likely that normalisation of the axis is related to complete remission between episodes, as opposed to incomplete remission.

Comparison of patients with Treatment Resistant Unipolar Depression and Treatment Resistant Bipolar Depression

CAR

The CAR was measured in subjects with TRBD and TRUD, and compared between these 2 groups. AUC_i and AUC_g were found to be higher in TRUD particularly on Day 1. Although this is the first time that the CAR has been compared between patients with TRUD and TRBD, on a similar note, a previous study revealed a higher AUC_i in subjects with a lifetime diagnosis of Unipolar Depression compared to those being in the Bipolar Spectrum. This study did not assess current symptomatology, however it is not clear in which phase of illness patients were, nevertheless it is the only study to have measured the AUC_i in patients with a previous diagnosis of Bipolar Disorder (Jabben et al., 2011).

Thus, it seems that a lower CAR is a finding in TRBD as well as Bipolar Spectrum Disorder, as opposed to Unipolar Disorder. In previous research, the only other condition shown to have an attenuated CAR during a depressive phase is Seasonal Affective Disorder (SAD). This study does not specify if the underlying condition is UD or BD (Thorn et al., 2011).

Explanation of results based on neurovegetative symptoms

Previous studies have shown that episodes of Bipolar Depression are more likely to involve reverse neurovegetative symptoms such as hyperphagia and hypersomnia (Perlis et al., 2006) and we also found the same when comparing the 2 groups (Chapter 3).

This is interesting as Atypical Depression, characterised by reversed neurovegetative features, has been previously linked to hypocortisolism (Gold and Chrousos, 1998). A recent paper describes Type II Bipolar Disorder as being within a continuum between Major Depression and Atypical Depression (Akiskal et al., 2007), whilst Atypical Depression has also been described as a variant of Bipolar Depression Type II (Perugi et al., 2011). If one were to accept that the above conditions share similarities in their clinical picture, it is not surprising that they may also share similar endocrine abnormalities.

The same may also be true for SAD, a subtype of Depression with atypical features. In SAD, patients experience extreme changes in mood across season and report the ‘atypical’ depressive symptoms of hypersomnia, lethargy, overeating and carbohydrate craving during winter months (Sher et al., 1999). Previous studies have shown attenuation of the CAR during winter in patients with the sub-type of SAD of depression, whereas the opposite has been shown in depressed patients without SAD, ie an enhanced CAR during winter months (Thorn et al., 2011). In our study we tried to test the hypothesis of whether the CAR is lower in the winter in subjects with TRBD similarly to SAD and if the opposite is true in TRUD. We did find that in TRUD patients have a higher CAR in the winter (not statistically significant). In TRBD,

secretion of cortisol post awakening was higher in the summer compared to winter on Day 1 (AUCg, $z=-2.19$, $p=0.02$, winter 6 patients median (IQR) 60.2 (11.4) nmol/l.h vs summer 3 patients 82.2 (32.7) nmol/l.h. Whilst there are intriguing preliminary data, our small numbers make it very difficult to draw any clear conclusions on the above.

Evidence from studies on hippocampal volume

A recent meta-analysis showed that hippocampal volume was larger in patients with Bipolar Disorder compared to Unipolar Disorder (Kempton et al., 2011). This is relevant to our study as a smaller hippocampal volume has been linked with hypercortisolemia. Thus, it is plausible that if patients with Bipolar Disorder have larger hippocampal volume compared to Unipolar Depression, and given that smaller hippocampi are linked to hypercortisolemia, this is consistent with the suggestion that Bipolar Depression, the predominant clinical state in Bipolar Disorder, is not associated with hypercortisolemia, and could be associated with hypocortisolemia as found in our study. Unfortunately, there are no neuroimaging studies of patients specifically with Atypical Depression, or comparing hypercortisolemic depression with non-hypercortisolemic depression, to test this more directly.

Summary

In summary, patients with TRBD were found to be hypocortisolemic compared to TRUD, as measured by the salivary CAR. This hypocortisolemia was more prominent on Day 1, possibly related to the novelty of the stimulus.

DHEA (only salient points are discussed below)

Comparison of patients with Treatment Resistant Unipolar Depression and controls

Daily secretion of DHEA did not differ between patients and controls. Daily secretion, as opposed to single concentrations of DHEA during the day, has never been previously measured in uncomplicated depression or TRUD, thus it is the first time that unaltered DHEA daily secretion is reported in depression. Equally DHEA secretion post awakening was not different between patients and controls on any of the 4 days, which is again a previously unreported finding. Furthermore, looking at DHEA concentrations post awakening in patients and controls we conclude that there is no ‘DHEA awakening response’, which is in line with previous studies (Hucklebridge et al., 2005).

Thus, we found no difference between patients and controls in DHEA levels on awakening or at any part of the day. This is in line with some previous studies using single DHEA measurements, which have shown unaltered levels of DHEA(S) in Unipolar Depression (Hsu et al., 2009). Thus, in TRUD the results using serial saliva measures replicate those using single plasma measures (Study I) suggesting that there seems to be no ‘compensation’ of antiglucocorticoid activity for the high cortisol levels.

In the current study subjects were not assessed following treatment, thus at this stage we are not able to comment as to whether salivary DHEA levels (concentrations) are lower in treatment responders compared to non responders at follow up, a finding reported in our pilot study using plasma DHEA.

In summary, DHEA secretion and daily DHEA concentrations did not differ between our groups. Furthermore, in line with previous studies, a ‘DHEA awakening response’ does not seem to exist (Hucklebridge et al., 2005).

Comparison of patients with Treatment Resistant Unipolar Depression, Treatment Resistant Unipolar Depression in remission and controls

DHEA secretion and concentration post awakening, or its daily secretion (Tables 11 and 14) was not different between the 2 patient groups, or between remitted patients and controls. The null finding regarding salivary DHEA is a novel finding in TRUD and has never been evaluated in depression. As also found in our plasma study, we were not able to see a change in DHEA levels. Thus, as in our plasma study if one were to accept that DHEA has an antiglucocorticoid activity as suggested (Bauer et al., 2002), it does not seem that DHEA changes in a similar way as cortisol to counteract the hypercortisolemia observed in TRUD.

Comparison of patients with Treatment Resistant Bipolar Depression, Treatment Resistant Bipolar Depression in remission and controls

Daily secretion of DHEA, measured for the first time in TRBD, did not differ between patients and controls. Secretion of DHEA has never been previously measured in Bipolar Depression, thus comparing our results with previous ones is not possible. However, post -mortem results have found higher DHEA concentrations in brain in Bipolar Disorder (Marx et al., 2006). We were not able to replicate this finding and thus we are not able to fully support the notion that DHEA as an antiglucocorticoid is increased either de novo in the brain or in the periphery. What we can report is that DHEA does not seem to change alongside cortisol. Thus, in TRBD, dysregulation of the

HPA axis is not balanced by a counteractive mechanism that might restore homeostasis of the low glucocorticoid levels observed and in particular the reduced CAR.

Cortisol/DHEA ratio (salient findings discussed below)

Comparison of patients with Treatment Resistant Unipolar Depression and controls

Single salivary Cortisol/DHEA concentration was higher in patients on Day 1 following awakening. Thus, in saliva, and as also shown in the plasma pilot study there is an excess of glucocorticoid activity possibly contributing to an increased allostatic load. Previous studies have found the salivary ratio to be elevated in depressed patients compared to controls (Young et al., 2002; Michael et al., 2000), a finding which also agrees with our preliminary pilot results, albeit on 1 of the 4 days measured. On the other hand, Cortisol/DHEA daily secretion was not found to differ between patients and controls on Day 1. On Day 2, it was higher in patients compared to controls both in the morning and during the day. One explanation of the excessive net glucocorticoid activity during some days and not others is that the body is trying to regulate itself by bringing cortisol levels down. This is not necessarily via the negative feedback mechanism, but may also be through other routes such as splanchnic nerve activation. Another explanation is that the excessive net glucocorticoid activity and higher Cortisol/DHEA ratio is particularly prominent at certain times of stress for the individual, which is when we see the decompensation of the HPA system.

Summary

In summary, the secretion of Cortisol/DHEA was consistently higher on Day 2. We were able to partially replicate the higher Cortisol/DHEA ratio found in our pilot study, but only during 1 of the 4 days measured. Although power issues may have masked differences on Day 3 and 4 in the saliva study, it is not clear why the ratio was higher on Day 2, as opposed to Day 1, but some explanations have been attempted. With regards to whether the Cortisol/DHEA ratio can be useful as a biomarker, given that we have previously established its stability compared to simply measuring cortisol and DHEA we conclude that more research is needed possibly with larger numbers to ascertain this.

Comparison of patients with Treatment Resistant Unipolar Depression, Treatment Resistant Unipolar Depression in remission and controls

The Cortisol/DHEA concentrations or secretion (Tables 12 and 15) were not different between remitted patients and controls, with the exception of a lower ratio in remitted patients compared to controls on Day 2 at noon. This may be a random finding, or it could be related to an attempt of the body to counteract the excessive glucocorticoid activity by causing a 'depletion'. Overall, however, it seems that during remission there is normalisation of the Cortisol/DHEA ratio as shown by a grossly stable ratio in remission. This finding was also found in the pilot study, thus, the ratio of Cortisol/DHEA did not differ between patients that responded to treatment and controls and it seems that the ratio of Cortisol/DHEA in remission is a stable marker.

Previous studies have shown that a high Cortisol/DHEA ratio predicted relapse (Goodyer et al., 2003). Follow up has not yet taken place or evaluated in this study, or

other studies in TRUD. Thus, it is not clear whether the salivary Cortisol/DHEA ratio has a role in predicting relapse in TRUD.

Comparison of patients with Treatment Resistant Bipolar Depression and controls

The ratio of Cortisol/DHEA post awakening was not different between patients and controls on Day 1, but it was lower in patients on Day 2. However, the ratio of Cortisol/DHEA during the day measured by dividing the total secretion of cortisol and that of DHEA during the day (AUCg) did not differ between subjects with TRBD and controls on Day 1, or on Day 2.

Thus, in TRBD as opposed to TRUD it seems that there is a net glucocorticoid 'depletion' at some time points or days, such as for example after waking on Day 2. This depletion may again not be complete, which means that dysregulation of the HPA axis may be periodic and may be related to the body's partial attempt to counteract the hypocortisolemia that was evident on Day 1 post awakening. Another explanation is that following a stressful event, which in the current paradigm may be that of simply undertaking the test, the body responds by causing a lower net glucocorticoid activity. However, since very few studies have been done in this area it is difficult to draw conclusions regarding the ratio of Cortisol/DHEA in this condition and possibly more research is needed in this area.

Based on the results of our pilot study it may be useful to measure DHEA in subjects that do and do not relapse in the future, although our current numbers in TRBD may not allow such a sub-analysis at present. This is because in the pilot study in TRUD we found that the ratio of Cortisol/DHEA is higher in patients who did respond to treatment

compared to non responders and controls, which may be related to the fact that responders have an illness, which is more biologically driven and less related to co-factors as psychological reasons. We do not yet know if the same holds for TRBD.

Comparison of patients with Treatment Resistant Bipolar Depression in remission and controls

The ratio of Cortisol/DHEA during the day (AUCg) was higher in subjects with TRBD in remission compared to controls on Day 1 and also on Day 2. The ratio of Cortisol/DHEA post awakening (AUCg) was higher in patients on Day 2.

This may mean that these patients have a more severe, enduring condition, which is also genetically determined, compared to patients with TRUD. These abnormalities as shown by an abnormal elevated ratio are possibly related to excess net glucocorticoid activity. This does not come as a surprise given that these patients function in a state of hypercortisolemia in remission compared to controls as also shown by a higher daily CAR in 2/4 days. The higher ratio may be a marker of vulnerability to illness and may be used as a biomarker in patients in remission.

Summary

We come to the preliminary conclusion that in TRBD in remission there are unlikely to be significant residual abnormalities in the HPA axis and no net glucocorticoid excess or depletion. However, since very few studies have been undertaken in this area it is difficult to draw conclusions regarding the ratio of Cortisol/DHEA in this condition and possibly more research is needed in this area.

Further attempts to explain our findings

PSS

In an attempt to explain the low CAR in TRBD, subjects were compared regarding their PSS score. Stress is described as a state of disharmony, or threatened homeostasis (Tsigos and Chrousos, 2002). What is however also of significance is that the effect of stress on the HPA axis depends both on the time of exposure to the stressful stimulus, as well as the chronicity of the stimulus. Regarding the latter, studies have shown that chronic stressful stimuli cause a downregulation and further suppression of cortisol to the DST test (Stein et al., 1997; Yehuda et al., 2003) and lower cortisol levels compared to those during acute stress (Heim et al., 2000; Miller et al. 2007). PSS scores have been found to correlate inversely with the CAR measured over 4 days in previous studies (Thorn et al., 2006). In the present study, the lower CAR found in TRBD compared to TRUD may be additionally explained by the fact that patients with Bipolar Depression had a statistically significant higher PSS score compared to TRUD (See Chapter 3). The PSS is measured over the last month, thus it cannot be classified as a measure of acute stress, but is more related to chronicity. In our study, however, PSS scores did not correlate to the CAR, suggesting that there was not a strong dose response effect of this underlying the observed differences between TRUD and TRBD.

Life events

The number of life events measured in our sample over the last 12 months differed between the 2 groups. Patients with TRBD described more life events compared to patients with TRUD, a finding which was statistically significant. Stress has been found to adversely affect the HPA axis and, although both acute and chronic stress is

associated with hyperactivity of the HPA axis, there are some cases where persistence of chronic stress leads to hypoactivity of the HPA axis (Hellhammer and Wade, 1993; Fries et al., 2005). Therefore although there have been reports of a heightened CAR in social stress, worrying and lack of social recognition, chronic work overload (Wust et al., 2000; Schlotz et al., 2004), as well as in caregivers with high levels of stress (De Vugt et al., 2005), there has also been recent evidence to suggest that a blunted CAR is related to chronic stress levels (Buchanan et al., 2004; Thorn et al., 2006). Thus, given there was a difference in stress levels between our 2 groups, with patients suffering from TRBD exhibiting more life events, and since chronic stress has been found to downregulate the HPA axis, this may be another potential factor contributing to the lower CAR in TRBD.

Other variables in subjects with Treatment Resistant Unipolar Depression

Most of the sociodemographic factors did not have an effect on the CAR in our study. This is not surprising as the ES of these factors has been found in previous studies to be small. Nevertheless, some factors did seem to have an effect on the CAR and these are discussed below:

Physical illness

Secretion of DHEA post awakening and during the day was found to be lower in patients with physical illness. This finding was shown for the first time in TRUD. Low concentrations of DHEA have been previously found in physical illness (Kalimi and Regelson, 2000).

Medication

Previous research has shown that medication can affect the HPA axis. For example, citalopram and escitalopram have been found to increase levels of cortisol in healthy volunteers (Nadeem et al., 2004; Bhagwagar et al., 2002). Reboxetine increases both plasma and salivary cortisol (Hill et al., 2003). When the CAR was assessed, patients on TCAs were found to have a flatter curve (AUC_i) compared to those using SSRIs or other antidepressants, although the AUC_g did not differ between the groups (Manthey et al., 2011).

Scharnholz and colleagues, as previously mentioned reported that venlafaxine did not attenuate morning cortisol, however lower evening cortisol was associated with treatment response (Scharnholz et al., 2010). Despite these findings, other than for TCAs as above, medication did not seem to have any significant effect on cortisol in our study. However, it is very likely that our numbers were too small for sub-analyses and that our study may not have been adequately powered to detect smaller effects of medication.

Regarding the effect of medication on DHEA, we found that only lithium treatment has an effect on DHEA. Although DHEA(S) lowers following chronic lithium treatment in rat brain (Maayan et al., 2004), DHEA(S) is not shown to be affected in humans by lithium treatment (Baptista et al., 1997). This is the first time that DHEA is found to be higher in subjects with TRUD that use lithium as an augmentation of their antidepressant treatment. This is interesting as lithium used in monotherapy has been shown in a previous study to increase ACTH and cortisol responses in the DEX/CRH test in drug naïve patients with a depressive episode and in patients with unipolar depression who used lithium as augmentation. Thus, it seems that lithium may work in

part by normalising the HPA axis or counteracting the effects of hypercortisolemia. In our study we could not find an effect of lithium on cortisol possibly related to the power of the study, however the upregulation of DHEA for the subjects that use lithium as an augmentation strategy in our sample may be related to the normalised ratio in remission.

Abuse

a. Cortisol

Although some studies have reported attenuated cortisol levels in victims of sexual abuse (Trickett et al., 2010), as well as lower salivary cortisol in response to TSST in those with a history of physical abuse (Carpenter et al., 2011), in depression, childhood abuse has been shown to be related to increased cortisol reactivity (Heim et al., 2000). Social anxiety had a similar effect on cortisol following a stress test (Elzinga et al., 2010). In our study, abuse did not have an appreciable effect on cortisol levels, which may be because it was not adequately powered to detect effects of abuse, or because abuse is not a major determinant of cortisol levels in treatment resistant affective disorders.

b. DHEA

Subjects that were physically abused had a higher DHEA following awakening on Day 1 and 2. They also had a higher daily secretion of DHEA on Day 1. Subjects that were sexually abused had a higher DHEA secretion following awakening on Day 1.

Similarly to the results in our study, daily plasma DHEA(S) concentration has been found to be higher in patients with PTSD related to childhood abuse. Salivary DHEA morning concentrations have been found to be higher in subjects with PTSD and

Borderline Personality Disorder (Jogems-Kosterman et al., 2007). Kellner and colleagues also found that DHEA(S) plasma levels increased in subjects with PTSD and childhood abuse (Kellner et al., 2010). Our results therefore seem to agree with previous research. Some previous studies suggested that the higher DHEA in victims of abuse may actually be a marker of resilience in that individuals who develop a mental illness because of experiencing abuse are more resilient compared to those who develop the illness in the absence of a risk factor such as abuse. Thus, the higher DHEA in subjects with abuse that developed depression may in fact be a marker of resilience compared to their counterparts (Yehuda and Flory, 2007).

Time of awakening

We measured cortisol levels and the CAR by performing a median split between late and early awakeners, as it has previously been shown that post-awakening cortisol is higher in early compared to late awakeners. We found that there was no difference between early and late awakeners on the CAR. Furthermore, secretion of cortisol during the day did not correlate with awakening time. Previous studies have shown that secretion of cortisol during the day is higher in early compared to late awakeners (Wust et al., 2000). Our numbers may have been too small to detect a possibly small effect of awakening time on the CAR. Furthermore neither the CAR, nor the daily cortisol secretion, correlated with the Pittsburgh Sleep Quality Index (PSQI), showing that sleep quality does not affect cortisol levels. Previously the CAR has been found not to relate to sleep duration when controlling for higher stress perception and impaired sleep quality, but a higher CAR has been linked to sleep disturbance (Williams et al., 2005).

Variables that did not have an effect on the CAR

Smoking

Smoking did not affect the CAR or cortisol and DHEA levels during the day. From previous epidemiological studies it seems that the ES of smoking on the CAR is small (Badrick et al., 2007) and that smoking does not always affect the CAR (see Chapter 1). This in addition to our small sample size may have been the reason behind our null finding.

Place of collection

Although this comparison was not always undertaken, as in some of the experiments all subjects collected from hospital, overall the CAR did not differ between patients who collected saliva whilst on the ward, or at home. The CAR has been previously shown to be similar in subjects collecting from home or under strict controlled lab conditions (Kudielka and Wust, 2010). This may also mean that undertaking the test inside the ward is not more stressful than at home. Factors such as staff support in a hospital environment and the relatively long duration of inpatient stay, may be the reasons behind this. It may also mean that in our sample admission status is not related to the severity of illness meaning that outpatients may have been as severely ill as inpatients.

Menstrual phase

Menstrual phase did not affect the CAR in any of the groups. Results from previous studies are conflicting, with some studies reporting that the salivary cortisol rise does not differ between menstrual phases (Kudielka et al., 2003) and other studies reporting that salivary responses differ significantly between women in the luteal or follicular phase when using the TSST psychological test (Kirschbaum et al., 1999). Most of the

subjects in our studies were post menopausal and possibly our study may not have been adequately powered to detect any effects of menstrual cycle that may have been present.

Day of collection

An analysis was conducted to determine whether there were hormonal differences between subjects collecting during weekdays and weekends. Neither the CAR, nor the AUCg for daily cortisol secretion were different between groups on either days. Some previous studies have found a higher CAR on a weekday compared to a weekend, a finding mostly related to work overload or worry (Schlotz et al., 2004). Since very few of our subjects were in employment, this may be the reason why cortisol did not seem to differ in our sample.

Gender

Gender did not seem to have an effect on the CAR, a finding which is in agreement with a recent review that concluded that the impact of gender on the CAR is small (Fries et al., 2009).

Variables in subjects with Treatment Resistant Bipolar Depression

Medication

Regarding mood stabilisers, DHEA-S levels have been found to be lower in females taking carbamazepine (Svalheim et al. 2009). Such medication is known to induce the P450 enzymes, metabolise DHEA(S) and thus decrease circulating concentrations of these hormones (Salek et al., 2002). However, we did not find any difference on DHEA levels between subjects who were and were not on carbamazepine, or other mood stabilisers. It seems likely that comparisons of individual drugs in this sample was

hampered by the high rates of multiple mood stabiliser use or fleeting the treatment resistant nature of their illness.

Limitations

Some limitations of studies II and III are listed below:

It would be important to examine the CAR in a larger sample. This is especially so for the TRBD group. Patients with TRBD are difficult to recruit and it took us almost 4 years to recruit the number of subjects with TRBD in this study. Additionally, given that the majority of patients were females, it would be informative to study a sample of equal numbers of females and males, as although we did not find an effect of gender on the CAR, female sex hormone levels may conceivably have affected our results.

We do feel that using subjects on medication is a limitation, given that many psychotropic medications may affect the HPA axis. However it is unlikely that this accounted for the differences found between TRUD and TRBD. Furthermore patients in remission were also on psychiatric medication nevertheless the HPA axis was normal in that case. Lastly, even if some of the observed differences reflect changes secondary to drugs, this may be still relevant to symptoms and indicated treatments in the two conditions.

In an attempt to understand whether the abnormalities found are related to the course of the illness or subsequent episodes, future studies may need to measure the above endocrine parameters during earlier stages of the illness. Thus, in order to establish whether hypercortisolemia in TRUD and hypocortisolemia in TRBD is a cause rather

than effect of the illness, that it that it is not subsequent to the episodes of the illness or an artifact of psychiatric medication, subjects will need to be assessed during the first illness episodes and ideally when they do not take medication. However there are several obstacles to undertaking such a study. The main difficulty lies in the fact that in Bipolar Disorder, as previously mentioned in this thesis, the time lag from the first episode to correct diagnosis may be up to ten years (Scott and Leboyer), by which time one might not know whether it is again the illness or the episodes that cause the HPA axis abnormalities. Furthermore during that time subjects often receive medication, as most of them have by that time received 1-4 diagnoses. In addition, during episodes of at least a moderate severity it is extremely difficult to withhold medication for ethical reasons. In uncomplicated episodes on the other hand, subjects may not have appreciable endocrine abnormalities on a biological level and large numbers of patients may be needed for the design of such a study.

When the study was initially designed a 4 day protocol, including measuring the CAR over 4 days was attempted. We did in fact pilot that protocol. However most of the subjects found it extremely difficult to adhere to a 4 day protocol with collection of samples over 8 time points. The idea of a full 4 day protocol was therefore consequently modified and for Days 3 and 4 we asked subjects to collect saliva only on awakening, noon and in the evening (3 time points). Some subjects however did not consent, or were not able to adhere to this modified protocol either, hence our numbers on Days 3 and 4 are smaller than Days 1 and 2. However using a 2 Day protocol is a strength in this study. This is a limitation to our study especially given that previous studies have insisted on a 4 day protocol when measuring cortisol levels, although not necessarily for the CAR (Goodyer et al., 2001). Since it is the first time that this study is attempted in

TRBD it may be interesting to assess the CAR and daily cortisol secretion over more days, especially given the degree of variability seen in our study.

Patients' adherence to the sampling protocol was evaluated by the use of the subject completed forms, as described in detail in the methodology chapter. There are other ways of assessing adherence, the advantages and disadvantages of which are described in Chapter 1. Because most of the subjects in our studies were recruited whilst on the ward, where they were monitored by staff, we do not feel that adherence to protocol is likely to have been a problem in our study. Specifically, we have no reason to believe this may have contributed to the lower CAR in the TRBD group on Day 1.

Summary of all studies and recommendations

In summary in these series of studies we measured Cortisol, DHEA and their ratio initially in a pilot study in plasma and compared responders and non responders. We showed that in plasma there is an excess of glucocorticoid activity as found by higher basal cortisol and a higher ratio of Cortisol/DHEA between patients and controls. We also showed that subsequent to treatment responders had a lower DHEA and a higher Cortisol/DHEA ratio on admission compared to non responders, which may be related to responders having an illness which is more biologically driven and hence more amenable to intensive pharmacological therapy. We then moved on to measure basal levels of cortisol as well as the CAR, DHEA and the ratio of Cortisol/DHEA, in a new sample of patients and using saliva rather than plasma. This study was done in TRUD, TRUD in remission, TRBD, TRBD in remission and controls. We were able to show that in subjects with TRUD there is an excess of glucocorticoid activity as shown by a

higher CAR and higher basal cortisol levels, whereas the opposite happens in subjects with TRBD where we showed that there is a lower CAR especially on Day 1, possibly related to novelty. We were able to explain the above finding by showing that patients with TRBD have more atypical symptoms as measured by the HAM-D 21 Item. The PSS, a measure of chronic stress, was also higher in these subjects. We did not find any difference in DHEA, suggesting that the dysregulated glucocorticoid activity is not counteracted by a higher or lower antiglucocorticoid activity. The ratio of Cortisol/DHEA was higher in TRUD during some days, suggesting that this excess net glucocorticoid activity is not complete but disjointed and possibly related to stressful stimuli.

We also assessed Cortisol, DHEA and their ratio during remission in both groups and found no consistent evidence for increased or decreased glucocorticoid or antiglucocorticoid activity. We therefore conclude that during remission the HPA axis returns to its normal level of functioning and it is important to continue treatment until a state of remission is achieved, even in treatment resistant affective disorders.

As mentioned, in the pilot plasma study we also found that responders to treatment have lower DHEA levels compared to non responders and this may be related to a more biological illness.

The most novel and important finding of this thesis is probably the low CAR in TRBD. This finding has important implications, although further research is needed in larger samples in order to replicate it, concentrating on the depressive state of Bipolar Disorder. Research should also concentrate on whether hypocortisolemia is a product of treatment resistance, perhaps due to chronicity of illness or propensity to experience or

respond to life events, or whether hypocortisolemia instead causes treatment resistance. If the latter is true, then novel treatment strategies may be indicated in TRBD. For example, in relation to pharmacological treatment, methods to increase HPA axis activity may be beneficial in TRBD as opposed to the existing strategies to decrease HPA activity that are sometimes used in unipolar depression. Indeed, it may be that HPA axis antagonists may worsen any hypocortisolemia in TRBD and hence need to be avoided in TRBD. Strategies to increase HPA axis could include steroid replacement. In this regard, there is preliminary evidence of the utility of low dose prednisolone in hypocortisolemic TRD (Bouwer et al., 2000). Other methods could include methods to increase endogenous steroid release, or direct glucocorticoid receptors agonists.

Also, patients with BD may respond to treatments that are used in SAD given the similarities between the 2 conditions, as previously mentioned in this thesis. One of these treatments may be the use of light therapy, often used in SAD. If Bipolar Depression is indeed classified under seasonal disorders then generally treatments to correct these abnormalities may be helpful. Agomelatine is a melatonin agonist and preliminary data show its efficacy in Bipolar Depression.

This may also be an important study on a clinical level given that differentiating between the 2 conditions (Unipolar vs Bipolar Depression) is often a challenge even by experienced clinicians. It may be that in these cases the CAR may serve as a biomarker, at least in patients that are treatment resistant. If future studies show a decreased CAR in uncomplicated Bipolar Disorder, then the CAR may be used as a biomarker towards diagnosis in earlier phases of depression. It may also be possible in future to categorise depression based on biomarkers such as the CAR, rather than longitudinal pattern, in

order to optimise choice of therapy. Patients with a salivary low CAR may be more likely to be diagnosed with BD as opposed to UD in complementing the clinical picture or in cases of clinical dilemmas.

Effects of treatment need to be looked at in terms of longer term effects on GR receptor function. There are certain medications that act on the 5HT2a system that are effective in lowering cortisol, such as mirtazapine and quetiapine. These medications, particularly their combination, may need to be avoided in TRBD (in our sample of TRBD these medications did not affect the CAR- 2 subjects). This is interesting as Quetiapine is the one of the 2 medications currently licensed for Bipolar Depression. Its long term effects especially on GR function have not been evaluated. This becomes of particular importance as Sanchez Gistau and colleagues have previously shown that atypicality in Bipolar Disorder, possibly related to hypocortisolemia, is related to suicide attempts (Sanchez-Gistau et al., 2009).

Finally, genetic research is very important in order to further clarify genetic abnormalities and the underlying core HPA axis abnormalities of treatment resistant affective disorders. Genetic research is particularly important in Bipolar Disorder, as this condition is more hereditary compared to Unipolar Disorder as also shown in twin studies. We believe that genetic research in Bipolar Disorder should concentrate on looking at abnormalities related to cortisol and possibly the SCN and hippocampus and receptors in these areas. Should genetic research be able to target the site of abnormalities then treatments could potentially target earlier stages of the disorder or even prevent the onset of illness episodes. Although perhaps optimistic, and in any

event still many years away, there is hope that genetic research may serve ultimately in eradicating this illness to the benefit of countless millions of people worldwide..

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APPENDIX 1.

HORMONE MEASUREMENT

Plasma and saliva measurements of hormonal concentrations

Study I, Treatment Resistant Unipolar Depression/plasma

Plasma cortisol concentrations were measured using the DSL-2100 “Active Cortisol” Radioimmunoassay (RIA) Kit (Diagnostic Systems Laboratories). Intrassay precision was 6% at 74nmol/L and 5% at 360 nmol/L. Minimal detectable concentration was 14 nmol/L.

Plasma DHEA was assayed in duplicate using a Luminescence immunoassay (IBL, Hamburg, Germany). If the difference (coefficient of variance – CV) between the duplicate measurements was higher than 10%, the analysis was repeated. The minimal detectable concentration was 0.4 nmol/L. Plasma specimens were diluted 1:41 with the kit’s diluent before analysis-

Study II, Treatment Resistant Unipolar Depression/saliva

Study III, Treatment Resistant Bipolar Depression/saliva

Saliva cortisol concentrations were determined using the chemiluminescence assay of ‘Immulite’ - DPC’s automated Immunoassay analyser. (www.diagnostic.siemens.com) The same test units (LCO1) and wedge reagents (LCO2) and substrate (LSUBX) were used as for the plasma cortisol assay (LKCO1) of the analyser. However

- a. the cortisol concentrations of unknown saliva specimens were read off a calibration graph constructed from 10 cortisol standards in saline- concn 0- 160 nmoles/L.
- b. the volume of the specimen taken for analysis was increased by 40U_L.

Multicalc v 2.65 (part no 1224-310; www.perkinelmer.co.uk) was used to plot the calibration graph and for immunoassay data processing.

The method correlated well with an in-house and previously published (Jurueña et al., 2006) TR-FIA ($r=0.94$, $y=0.004+1.08x$, $N=41$), had analytical sensitivity of 0.2 nm/l, mean % recovery of cortisol of 106.1 (range of cortisol concentrations from 5 to 65 nm/l). and inter/intra-assay precision (% CV) of less than 10% (range 5 to 25 nm/l).

The linearity upon dilution test (parallelism) resulted in a straight line ($r=0.99$, $y=0.144+1.014x$). The calibration graph was highly reproducible ($n=11$ assays) with slope (mean \pm sem) of 0.197 ± 0.004 .

The percentage cross reactivity of the antiserum with cortisone was 0.35%.

The short and long term performance of the assay was monitored with

- a. Biorad commercial control sera (www.bio-rad.com)
- b. Human saliva pools and
- c. Participation in external QC scheme for saliva steroids organised by IBL (www.ibl-hamburg.com)

Saliva DHEA concentrations were measured using an IBL (IBL, Hamburg, Germany) chemiluminescence immunoassay (product code RE62051).

Saliva specimens collected at each time point were defrosted, mixed and after centrifugation at 3500 rpm at room temperature, were treated as described below.

Briefly using the Genesis 100 Robotic Sample processor (Tecan UK, Theale, Reading, UK), 50 μ L of the test saliva or standard was added to the wells of microtitration strips. This was followed by 50 μ L of a solution of the enzyme labelled hormone and 50 μ L of the hormone antibody. Both of these reagents were added using an electronic Eppendorf Repette.

After an incubation of 4hrs at room temperature, the incubation solution was discarded and the wells were washed 4 times with 250 μ L of wash out buffer and 50 μ L of chemiluminescence reagent added. The luminescence of the bound fraction was measured in a Berthold Luminometer (MPLI, Berthold Detection Systems, Pforzheim, Germany) which was linked to MicroWin 2000 Version 4 (Microtek, Laborsysteme, Ovoroth, Germany) for immunoassay data processing. Hormone concentrations were read off a calibration graph, constructed from a series of seven DHEA standards (0, 12, 3, 37, 111, 333, 1000, 3000 pg/ml). The day to day performance of the assays was monitored using a) the kit's saliva control specimens b) the commercial control sera (Immunoassays- plus Biorad, Hemel Hempstead, Herts, UK) which had been suitably diluted with each kit's zero standard c) participation in an International QC scheme for saliva hormone Steroids organized by IBL.

Inter and Intra assay precision (%CV) of the saliva DHEA assay was about 15% at concentrations of < 60 pg/ml and below 10 % at concentrations above ca 400 pg/ml.

DHEA concentration units in pg/ml were multiplied by 0.0034 to convert them to nmoles/l.

APPENDIX 2.

Detailed Instructions for Saliva Collection

We ask you to collect your saliva on 4 consecutive days or if not then as close to each other as possible.

You can decide which days.

Choose the days when you are in good health and have no heavy social commitments ie late evenings, parties, social gatherings, entertainment and so on.

We will provide you with 4 small plastic bags. These will be labelled as DAY 1, DAY 2, DAY 3, DAY 4. They will contain the tubes you need to collect your specimens in. All tubes are labelled on the outside and numbered at the top.

Each day we ask you to collect your saliva immediately upon waking up (whichever time that is) and then at +15 mins, +30 mins, +45 mins, +60 mins, +90 mins and before your lunch. We also ask you to collect an evening specimen at 10 pm, a total of 8 specimens.

We will give you a form to fill in. Please complete this every time you collect your specimen inserting the date, time, you woke up and collected the specimens or telling us something about the events that took place the hour before you collected the specimens.

If you have any questions or need help you can contact myself Dr Kalypso Markopoulou.

e-mail address: Kalypso.Markopoulou@iop.kcl.ac.uk

DAY 1,2,3,4

Morning specimens

Wake up between 6 and 9:30 am and take the tubes from the bag labelled DAY 1,2,3or 4 according to the days you are collecting.

Do not brush your teeth and do not have breakfast or any other drinks. You can clear your throat before starting to collect specimens.

Sit by your bed or choose another quiet part of your bedroom and make a note on the collection form of the exact time you woke up eg 7.15 am. Start collecting your specimens in the tubes provided closing them firmly after each collection and noting the time on the collection form. Remember you need to fill each tube with clear saliva at least up to the 1 ml mark and above.

Take tube No 1 (labelled immediately on wake up) and collect your saliva.

15 minutes after waking up take tube no 2 and collect your saliva. Note the time on the collection form.

30 minutes after waking up take tube no 3 and repeat the saliva collection.

45 minutes after waking up take tube no 4 repeat the saliva collection.

60 minutes after waking up take tube no 5 repeat the saliva collection.

90 minutes after waking up take tube no 6 repeat the saliva collection.

Return your tubes into the plastic bag labelled DAY 1, 2, 3 or 4 and store in the freezer/leave your room.

Throw away your plastic straws once you have finished using them.

You can have breakfast now and continue with your other normal daily activities.

Before your lunch take tube no 7 and repeat the saliva collection.

Evening specimen

At 10 pm choose a quiet part to stay and preferably sitting down and take the tube labelled 10 pm and collect your saliva. Make a note on the collection form of the exact time eg 10.15 pm that you collected the specimen.

Return the tube to the bag labelled DAY 1 and leave in the freezer/your room.

Remember it is important that in the HOUR before giving the specimen you should try and avoid the following as these will affect the hormone measurements.

Eating or drinking anything- if you do have something accidentally you must make a note of it.

Demanding social activities ie late night parties/entertainments or any hassles eg arguments with friends/relatives, difficult conversations. If you can not then make a note on the collection sheet.

You should make a note if you are in any kind of pain (headache, migraine, toothache, backache) or feeling sick for some reason.

**IMPORTANT POINTS TO HELP YOU COLLECT ENOUGH SALIVA WITH THE
“STRAW AND TUBE METHOD”**

Collect enough saliva in your mouth.

Take the appropriate tube and plastic straw out of the labelled bag, remove the cap and place one end of the straw in your mouth and the other end low down, near the bottom of the collection tube.

Slowly pass the saliva/spit through the straw to the tube- do not blow otherwise this will cause frothing. Tap gently the bottom of the tube on a table top a few times – this helps a clear layer of saliva collect at the bottom of the tube and reduces “frothing”.

Move the end of the straw above the surface of the collected saliva in the tube and repeat the process until you have collected enough – well above the 1 ml mark on the tube.

Close the tube tightly, put it in the appropriate bag and leave it in the freezer/your room.

Remember

Morning specimens- these should be collected before you brush your teeth and before you have any breakfast or other drinks.

Evening specimens- Have your evening meal early. Do not have anything to drink or eat or chew gum or smoke about 1 hour before giving the specimen.

To make a note on the collection forms the time you collect each specimen and also if you experience any hassles (eg difficult conversations, arguments with friends/relatives) pain of any kind eg headache, migraine, toothache.

Post all your specimens to the National Affective Disorders Unit. We shall provide you with an envelope addressed to the unit.

STUDY DAY 1

Subject Code= Patient/Control

Name Age

Date

PLEASE DO NOT BRUSH YOUR TEETH EAT OR DRINK ANYTHING FOR AT LEAST ONE HOUR BEFORE THE COLLECTION. IF YOU DO HAVE TO DRINK WATER PLEASE DO IT IMMEDIATELY AFTER THE COLLECTION OF THE SAMPLES

Tube No	Sample	Time given	Comment	Lab Code
1	Immediately on waking up		Where were you? How were you feeling? What were you doing before the collection of the samples? Did you accidentally eat or drink?	
2	Waking+15 minutes		Where were you? How were you feeling? What were you doing before the collection of the samples? Did you accidentally eat or drink?	
3	Waking+30 minutes		Where were you? How were you feeling? What were you doing before the collection of the samples? Did you accidentally eat or drink?	
4	Waking+45 minutes		Where were you? How were you feeling? What were you doing before the collection of the samples? Did you accidentally eat or drink?	

5 Waking+60
 minutes

Where were you? How were you feeling?

What were you doing before the collection of the samples?

Did you accidentally eat or drink?

6 Waking+90
 minutes

Where were you? How were you feeling?

What were you doing before the collection of the samples?

Did you accidentally eat or drink?

7 Midday

Where were you? How were you feeling?

What were you doing before the collection of the samples?

Did you accidentally eat or drink?

8 10 pm

Where were you? How were you feeling?

What were you doing?

Did you accidentally eat or drink?

What were you doing before the collection of the samples?

Did you eat or drink?

STUDY DAY 2

Subject Code= Patient/Control

Name Age

Date

PLEASE DO NOT BRUSH YOUR TEETH EAT OR DRINK ANYTHING FOR AT LEAST ONE HOUR BEFORE THE COLLECTION. IF YOU DO HAVE TO DRINK WATER PLEASE DO IT IMMEDIATELY AFTER THE COLLECTION OF THE SAMPLES

Tube No	Sample	Time given	Comment	Lab Code
1	Immediately on waking up		Where were you? How were you feeling? What were you doing before the collection of the samples? Did you accidentally eat or drink?	
2	Waking+15 minutes		Where were you? How were you feeling? What were you doing before the collection of the samples? Did you accidentally eat or drink?	
3	Waking+30 minutes		Where were you? How were you feeling? What were you doing before the collection of the samples? Did you accidentally eat or drink?	
4	Waking+45 minutes		Where were you? How were you feeling? What were you doing before the collection of the samples?	

Did you accidentally eat or drink?		
5	Waking+60 minutes	<p>Where were you? How were you feeling?</p> <p>What were you doing before the collection of the samples?</p> <p>Did you accidentally eat or drink?</p>
6	Waking+90 minutes	<p>Where were you? How were you feeling?</p> <p>What were you doing before the collection of the samples?</p> <p>Did you accidentally eat or drink?</p>
7	Midday	<p>Where were you? How were you feeling?</p> <p>What were you doing before the collection of the samples?</p> <p>Did you accidentally eat or drink?</p>
8	10 pm	<p>Where were you? How were you feeling?</p> <p>What were you doing?</p> <p>Did you accidentally eat or drink?</p> <p>What were you doing before the collection of the samples?</p> <p>Did you eat or drink?</p>

STUDY DAY 3

Subject Code= Patient/Control

Name Age

Date

PLEASE DO NOT BRUSH YOUR TEETH EAT OR DRINK ANYTHING FOR AT LEAST ONE HOUR BEFORE THE COLLECTION. IF YOU DO HAVE TO DRINK WATER PLEASE DO IT IMMEDIATELY AFTER THE COLLECTION OF THE SAMPLES

Tube No	Sample	Time given	Comment	Lab Code
1	Immediately on waking up		Where were you? How were you feeling?	
			What were you doing before the collection of the samples?	
			Did you accidentally eat or drink?	
7	Midday		Where were you? How were you feeling?	
			What were you doing before the collection of the samples?	
			Did you accidentally eat or drink?	
8	10 pm		Where were you? How were you feeling?	
			What were you doing?	
			Did you accidentally eat or drink?	
			What were you doing before the collection of the samples?	
			Did you eat or drink?	

STUDY DAY 4

Subject Code= Patient/Control

Name Age

Date

PLEASE DO NOT BRUSH YOUR TEETH EAT OR DRINK ANYTHING FOR AT LEAST ONE HOUR BEFORE THE COLLECTION. IF YOU DO HAVE TO DRINK WATER PLEASE DO IT IMMEDIATELY AFTER THE COLLECTION OF THE SAMPLES

Tube No	Sample	Time given	Comment	Lab Code
1	Immediately on waking up		Where were you? How were you feeling? What were you doing before the collection of the samples? Did you accidentally eat or drink?	
7	Midday		Where were you? How were you feeling? What were you doing before the collection of the samples? Did you accidentally eat or drink?	
8	10 pm		Where were you? How were you feeling? What were you doing? Did you accidentally eat or drink? What were you doing before the collection of the samples? Did you eat or drink?	

Perceived Stress Scale

The questions in this scale ask you about your feelings and thoughts **during the last month**. In

each case, you will be asked to indicate by circling *how often* you felt or thought a certain way.

Name _____

Date _____

Age _____ Gender (*Circle*): **M F** Other _____

0 = Never 1 = Almost Never 2 = Sometimes 3 = Fairly Often 4 = Very Often

1. In the last month, how often have you been upset because of something that happened unexpectedly? **0 1 2 3 4**
 2. In the last month, how often have you felt that you were unable to control the important things in your life? **0 1 2 3 4**
 3. In the last month, how often have you felt nervous and "stressed"? **0 1 2 3 4**
 4. In the last month, how often have you felt confident about your ability to handle your personal problems? **0 1 2 3 4**
 5. In the last month, how often have you felt that things were going your way? **0 1 2 3 4**
 6. In the last month, how often have you found that you could not cope with all the things that you had to do? **0 1 2 3 4**
 7. In the last month, how often have you been able to control irritations in your life? **0 1 2 3 4**
 8. In the last month, how often have you felt that you were on top of things?.. **0 1 2 3 4**
 9. In the last month, how often have you been angered because of things that were outside of your control?..... **0 1 2 3 4**
 10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them? **0 1 2 3 4**
- Please feel free to use the *Perceived Stress Scale* for your research.

References

The PSS Scale is reprinted with permission of the American Sociological Association, from Cohen, S., Kamarck, T., and Mermelstein, R. (1983). A global measure of perceived stress. *Journal of Health and Social Behavior*, 24, 386-396.
Cohen, S. and Williamson, G. Perceived Stress in a Probability Sample of the United States. Spacapan, S. and Oskamp, S. (Eds.) *The Social Psychology of*

CORTISOL/DHEA IN DEPRESSION

Name:	CONTROL-TRD-MD-BD RECOVERED – BD -MD
Date of Birth:	Laboratory Code:
Sex: M/F	Hospital Number:
Weight & Height:	BMI:
Occupation	Ethnicity
DATE OF TEST Pre- / Post-Menopausal Menstrual Cycle	

Psychiatric Diagnosis:

General Physical Health/ Other Illnesses

Allergies/Pets

Smoking Habits

Any special Diets

Oral Hygiene

General Health Questionnaire: Y/N

Life Events (including recently):

Medication at time of testing

Psychiatric

Other

Steroids/Oral contraceptives

Intrusive Memory Protocol (Life Events)

1. *'Have you ever experienced:*

- a. Serious illness or injury? YES / NO if yes, when _____
(12mths preceding current episode?) _____
- b. The serious illness of a close relative? YES / NO if yes, when _____
(12mths preceding current episode?) _____
- c. The death of a close relative, such as
your child or husband/wife? YES / NO if yes, when _____
(12mths preceding current episode?) _____
- d. The death of a close family friend or a
second-degree relative? YES / NO if yes, when _____
(12mths preceding current episode?) _____
- e. Separation due to marital difficulties? YES / NO if yes, when _____
(12mths preceding current episode?) _____
- f. The break up of a steady relationship? YES / NO if yes, when _____
(12mths preceding current episode?) _____
- g. A serious problem with a close friend,
neighbour or relative? YES / NO if yes, when _____
(12mths preceding current episode?) _____
- h. Unemployment/job-seeking for more
than one month? YES / NO if yes, when _____
(12mths preceding current episode?) _____
- i. Being sacked from your job? YES / NO if yes, when _____
(12mths preceding current episode?) _____
- j. A major financial crisis? YES / NO if yes, when _____
(12mths preceding current episode?) _____
- k. Any problems with the police and a
court appearance? YES / NO if yes, when _____
(12mths preceding current episode?) _____

1. Having something valuable lost or
stolen?' YES / NO if yes, when _____

(12mths preceding current episode?) _____

2. *'Were there any other events that could have triggered the depression? Were there any other events that occurred before the depression?'*

3. *'How was your childhood? Was it happy? Were your carers loving? Were they strict? Were you harshly disciplined? Do you feel that you were well cared after? Did you have any unwanted sexual experiences? Have you ever been physically assaulted?'*

4. *'Have you had any memories of any of these events come spontaneously into your mind during the past week?'* (to count as intrusive, memories need to be a visual image of a specific scene that had actually taken place).

If so, *'can you describe these memories for me?'*

If more than two *'describe the most intrusive of these memories.'*

5. *'Have you experienced any other recurring memories of unpleasant events in the past week?'*

Intrusions rating - IES

- If no memories = 0
- If 1 memory:
Intrusions____, Avoidance ____, Total ____
- If 2 memories:
Memory 1: Intrusions ____, Avoidance, ____, Total ____

Memory 2: Intrusions ____, Avoidance, ____, Total ____

Impact of Events Scale

Below is a list of comments made by people after stressful life events. Please check each item, indicating how frequently these comments were true for you **DURING THE PAST SEVEN DAYS**. If they did not occur during that time, please mark the 'not at all' column.

Not at all Rarely Sometimes Often

1. I thought about it when I didn't mean to
2. I avoided letting myself get upset when I thought about it or was reminded of it
3. I tried to remove it from memory
4. I had trouble falling asleep or staying asleep because of pictures or thoughts about it that came into my mind
5. I had waves of strong feeling about it
6. I had dreams about it
7. I stayed away from reminders of it
8. I felt as if it hadn't happened or wasn't real
9. I tried not to talk about it
10. Pictures about it popped into my mind

11. Other things kept making me think about it

12. I was aware that I still had a lot of feelings about it, but I didn't deal with them

13. I tried not to think about it

14. Any reminder brought back feelings about it

15. My feelings about it were kind of numb

SF-12 v2 Health and Well-Being

For each of the following questions, please circle or X the best possible answer.

1. In general, would you say your health is:

Excellent Very Good Good Fair Poor

2.) The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

Yes, Limited A Lot	Yes, Limited A little	No, Not Limited At All
-----------------------------------	--------------------------------------	---------------------------------------

Moderate activities, such as moving a table,
pushing a vacuum cleaner, bowling, or playing golf

Climbing several flights of stairs

3.) During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities **as a result of your physical health?**

All of the time	Most of the time	Some of the time	A little of the time	None of the time
--------------------------------	-----------------------------	---------------------------------	---	---------------------------------

Accomplished less than you would like

Were limited in the kind of work or other activities

4.) During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?

All of the time	Most of the time	Some of the time	A little of the time	None of the time
--------------------------------	-----------------------------	---------------------------------	---	---------------------------------

Accomplished less than you would like

Were limited in the kind of work or other activities

5.) During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

Not at all A little bit Moderately Quite a bit Extremely

6.) These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks . . .

All of the time	Most of the time	Some of the time	A little of the time	None of the time
--------------------------------	-----------------------------	---------------------------------	---	---------------------------------

Have you felt calm and peaceful?

Did you have a lot of energy?

Have you felt downhearted and depressed?

7.) During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?

**All of Most of Some of A little of None of
the time the time the time the time the time**

PITTSBURGH SLEEP QUALITY INDEX (PSQI)

Name _____ ID# _____ Date _____ Age _____

Instructions:

The following questions relate to your usual sleep habits during the past month ONLY. Your answers

should indicate the most accurate reply for the majority of days and nights in the past month.

Please answer all questions.

1. During the past month, when have you usually gone to bed at night?

USUAL BED TIME _____

2. During the past month, how long (in minutes) has it usually taken you to fall asleep each night?

NUMBER OF MINUTES _____

3. During the past month, when have you usually gotten up in the morning?

USUAL GETTING UP TIME _____

4. During the past month, how many hours of *actual sleep* did you get at night? (This may be

different than the number of hours you spend in bed.)

HOURS OF SLEEP PER NIGHT _____

For each of the remaining questions, check the one best response. Please answer *all* questions.

5. During the past month, how often have you had trouble sleeping because you.....

(a) cannot get to sleep within 30 minutes

Not during the Less than Once or Three or more

past month _____ once a week _____ twice a week _____ times a week _____

(b) Wake up in the middle of the night or early morning

Not during the Less than Once or Three or more

past month _____ once a week _____ twice a week _____ times a week _____

(c) Have to get up to use the bathroom.

Not during the Less than Once or Three or more

past month _____ once a week _____ twice a week _____ times a week _____

(d) Cannot breathe comfortably.

Not during the Less than Once or Three or more

past month _____ once a week _____ twice a week _____ times a week _____

(e) Cough or snore loudly.

Not during the Less than Once or Three or more

past month _____ once a week _____ twice a week _____ times a week _____

(f) Feel too cold.

Not during the Less than Once or Three or more

past month _____ once a week _____ twice a week _____ times a week _____

(g) Feel too hot.

Not during the Less than Once or Three or more

Past month _____ once a week _____ twice a week _____ times a week _____

(h) Had bad dreams.

Not during the Less than Once or Three or more

Past month _____ once a week _____ twice a week _____ times a week _____

(i) Have pain.

Not during the Less than Once or Three or more

Past month _____ once a week _____ twice a week _____ times a week _____

(j) Other reason(s), please describe _____

How often during the past month have you had trouble sleeping because of this?

Not during the Less than Once or Three or more

Past month _____ once a week _____ twice a week _____ times a week _____

6. During the past month, how would you rate your sleep quality overall?

Very good _____

Fairly good _____

Fairly bad _____

Very bad _____

7. During the past month, how often have you taken medicine (Prescribed or "over the counter") to help you sleep?

Not during the Less than Once or Three or more

Past month _____ once a week _____ twice a week _____ times a week _____

8. During the past month, how often have you had trouble staying awake while driving, eating

meals, or engaging in social activity?

Not during the Less than Once or Three or more

Past month _____ once a week _____ twice a week _____ times a week _____

9. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?

No problem at all _____

Only a very slight problem _____

Somewhat of a problem _____

A very big problem _____

10. Do you have a bed partner or share a room?

No bed partner or do not share a room _____

Partner/ flatmate in other room _____

Partner in same room, but not same bed _____

Partner in same bed _____

11. If you have a bed partner or share a room, ask him/her how often in the past month you

have had.....

(a) Loud snoring.

Not during the Less than Once or Three or more

Past month _____ once a week _____ twice a week _____ times a week _____

(b) Long pauses between breaths while asleep.

Not during the Less than Once or Three or more

Past month _____ once a week _____ twice a week _____ times a week _____

(c) Legs twitching or jerking while you sleep.

Not during the Less than Once or Three or more

Past month _____ once a week _____ twice a week _____ times a week _____

(d) Episodes of disorientation or confusion during sleep.

Not during the Less than Once or Three or more

Past month _____ once a week _____ twice a week _____ times a week _____

(e) Other restlessness while you sleep: please describe _____

—

Not during the Less than Once or Three or more

Past month _____ once a week _____ twice a week _____ times a week _____

[Buysse DJ, Reynolds CF, Monk TH, Berman SR, DJ Kupfer (1989) The Pittsburgh Sleep Quality Index: A New Instrument for Psychiatric Practice and Research, **Psychiatry Research**, 28: 193-213].